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Rates of Gastric Emptying in Young Cod

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Stomachs of young cod were opened at various time intervals after they had voluntarily eaten measured meals. More digested food left the stomach in the first few hours after the meal was given than towards the end of digestion. Instantaneous depletion rate increased rapidly with increase in acclimation temperature from 2 to 15 C. The rate was not increased at 19 C and was possibly depressed. Fish would not feed at 21 C. When meal size was varied, a straight-line relation was found between rate of gastric emptying and quantity of food in the stomach. This implies that in young cod intestinal absorption is coupled to food intake by regulation of the gastric volume. It is likely that digestion rate data could be used to estimate daily rations for fish in their own environment by measuring quantity of food in the stomach.

Received December 29, 1969

INTRODUCTION

THIS STUDY EXAMINES rates of gastric emptying in young cod (*Gadus morhua* L.) in relation to temperature and meal size. The work was undertaken as part of an effort to determine rates of energy passage within certain pathways of a marine food web. The relevance of digestion rate information in studies of natural production systems has been recognized by several workers, and summarized by Windell (1966). The primary information required is rate of gastric emptying in terms of calories, or milligrams of nitrogen, or other food component such as dry weight. On the average, the rate of gastric emptying will equal the rate of ingestion, and so a daily ration might be estimated for free fish in their natural environment. Such an estimate could not be made without information on effects of temperature, meal size, and characteristics of prey on digestion rate. The present paper supplies some of the information necessary for feeding rate estimates.

METHODS

Cod were caught by otter trawl in Passamaquoddy Bay, N.B. The fish were in their 2nd year of growth and averaged 29 cm long (range 23–35 cm) and 229 g (range 150–375 g), or 51.2 g dry weight. They were maintained by feeding every 2nd day with a ground mixture of beef liver, cod fillets, and Purina Trout Chow.

Prior to an experiment, fish were placed singly in fiberglass tanks of 75-liter capacity that were each supplied running salt water at a salinity of 28–30‰. Fifty-three fish were divided among acclimation temperatures of 2, 5, 10, 15, and 19 C. Tank temperatures were altered from the bay temperature to the acclimation temperature at a rate of 1 degree C every 2nd day. Fish were held at their acclimation level at least 2 weeks after the level had been reached. They were fed during the acclimation period and were not used in experiments until they fed well voluntarily. Fish would not feed when acclimated to 21 C.

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Cod were held in a closed room with simulated natural photoperiod during acclimation and experiments. Incandescent lights were controlled through a relay system with a photocell mounted out of doors.

Following the acclimation period the fish were not fed for a period of 3 days before the experimental meal if acclimated to 10, 15, or 19 C, or 4 days if acclimated to 2 or 5 C. The period of fasting allowed their stomachs to clear. They were then fed weighed quantities of shrimp tails (*Pandalus montagui*) cut into pieces of about $\frac{1}{4}$ inch in diameter without removal of the shell. Each piece had a volume of 0.6–0.8 ml, a wet weight of 0.45–0.64 g, and a dry weight of 0.10–0.16 g. The shrimp were caught in Passamaquoddy Bay and deep frozen (–18 C) within a few hours of capture.

A method of measuring meal sizes was chosen that would circumvent the problem of variations of water quantity on and within the shrimp pieces. Before a fish was fed, the thawed shrimp pieces were weighed under fresh water within a submerged dipper that was suspended from the pan of a magnetically dampened balance. Since the shrimp were heavier than water, the resultant weight was essentially equal to the dry weight minus the buoyant force. The submerged weight was multiplied by a correction factor so that the meal size could be expressed as dry weight. A correction factor was determined for each experiment by finding both the submerged weight and the dry weight of a quantity of shrimp prepared in the same way as shrimp fed to the cod.

The experimental meal was three pieces of shrimp and contained 0.40 g dry weight (.8% of the fish weight on a wet food–wet fish weight basis). The meal was given within a 15-min period. Fish stomachs were opened at various times after the meal and the stomach contents dried to constant weight, first in an oven for 5 hr at 50 C and then in a calcium sulfate desiccator for 4–8 days (depending on quantity of contents).

Experiments were repeated at each acclimation level except that sampling times were often altered so that data would be spread over the entire response period.

RESULTS

Curvilinear Depletion Curve

The relation between quantity remaining in the stomach and time interval after feeding was not linear (Fig. 1). Passage of chyme from the stomach to the intestine apparently occurred as rapidly as food was digested, since no liquid build-up was ever observed in the stomachs; that is, depletion rate paralleled digestion rate. Both rectilinear and curvilinear regressions were performed by computer on the data. The equations fit were

$$W = W_0 + bt \tag{1}$$

$$W = W_0 e^{-bt} \tag{2}$$

where W = dry weight remaining in the stomach (grams), W_0 = initial weight, b = regression coefficient, and t = time after feeding. The negative exponential fit gave reduced root mean square residuals for each of the acclimation levels as compared with the rectilinear fit, except for 15 and 19 C, where the residuals were about the same as the rectilinear fit. The curvilinear mean residuals and the rectilinear mean residuals (in parentheses) for combined replicates are as follows: 2 C, 0.03 (0.04); 5 C, 0.03 (0.05); 10 C, 0.03 (0.07); 15 C, 0.03 (0.03); and 19 C, 0.04 (0.03). The iterative method used in the curvilinear fit is described by Draper and Smith (1966). Some discussion may be found in Snedecor and Cochran (1967).

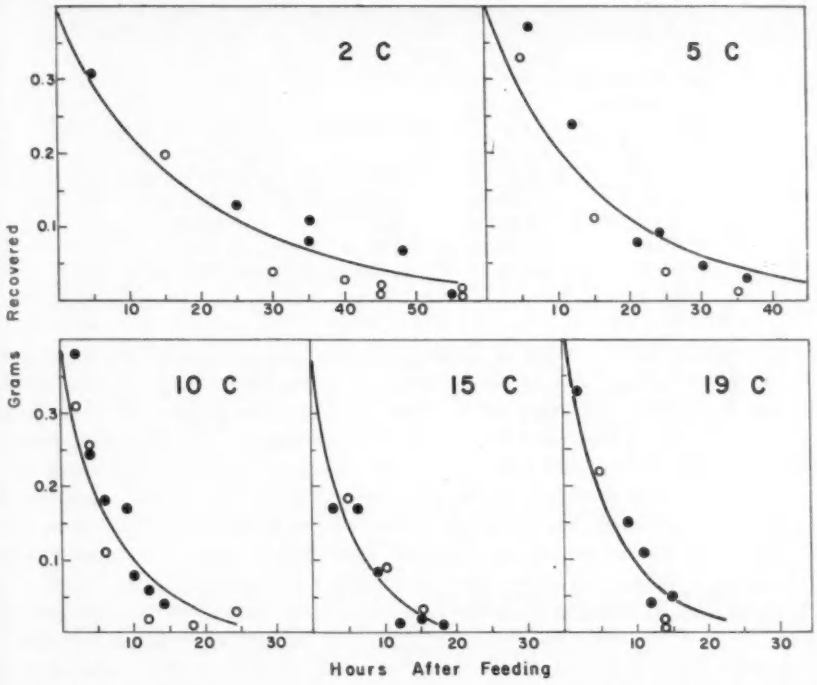

FIG. 1. Depletion of shrimp from cod stomachs at different temperature acclimations. Each point represents the dry weight of shrimp recovered from a fish. Open and solid circles within a panel indicate repeated experiments. Curves are fitted negative exponentials.

The data at 5 C seemed to show a lag of a few hours before digestion rate quickened. However, this lag was assumed to be an artifact, since it failed to show up at other acclimations. Subsequently, it was considered that a better estimate of rate of emptying would be achieved by fitting a line (equation 2) through the known meal size (fitting slope only) than by fitting both slope and Y intercept. The random error associated with the regression does not apply to the quantity of food fed, which is the Y intercept.

The regression coefficients of curvilinear fits to data from experiments repeated within acclimation levels were tested with a *t*-test (Wine, 1964) without finding significant differences at $P = 0.05$ (approximate; probability levels cannot be strictly assigned with curvilinear data since it is not possible to make unbiased estimates of population variance with existing technique (Draper and Smith, 1966)). Regressions were rerun on data combined within acclimation temperature. The fitted lines are drawn in Fig. 1. Values for model parameters are given in Table 1.

No significant differences could be detected in regression coefficients between different sizes of fish. Within each acclimation level, data were separated into two groups by weight of fish. The mean weights of the groups differed

TABLE 1. Fitted constants to the equation $W = W_0 \cdot \exp(B \cdot T)$ with approximate 95% confidence intervals at each acclimation temperature.

Temp (C)	W_0	B with fitted A	B with A at 0.40
2	0.41±0.09	−0.052±0.012	−0.051±0.006
5	0.57±0.13	−0.089±0.024	−0.063±0.015
10	0.50±0.10	−0.183±0.040	−0.143±0.025
15	0.33±0.15	−0.153±0.078	−0.184±0.034
19	0.47±0.12	−0.159±0.047	−0.139±0.030

by as much as 136 g within acclimation levels. Separate curves were fitted to the depletion data of each of the groups, and regression coefficients were tested by *t*-test within acclimation levels. Probabilities were all greater than 20%.

The curvilinear nature of the stomach-depletion curve means that greater quantities of digested food move out of the stomach in the first few hours than towards the end of digestion. From the fitted curves in Fig. 1, one can estimate average quantities of food leaving the stomach at different time intervals. During the first 5-hr period after feeding, quantities leaving the stomach were 0.10 g dry weight (25% of the meal given) at 2 C, 0.11 g (27%) at 5 C, 0.21 g (52%) at 10 C, 0.25 g (63%) at 15 C, and 0.20 g (50%) at 19 C. In the interval from 10 to 15 hr after feeding, the quantities passed at these temperatures were only 0.06 (15%), 0.06 (15%), 0.05 (13%), 0.04 (10%), and 0.05 g (13%), respectively.

If fish were allowed to feed continuously, what would their stomach depletion rates yield in terms of daily ration? Expressed as wet weight of the food and fish (229 g average fish weight), the mean rate of depletion in the first 5 hr, extrapolated to a 24-hr basis, would yield a daily ration of 0.87, 0.96, 1.83, 2.18, and 1.74% of the body weight at 2, 5, 10, 15, and 19 C, respectively. In terms of dry weight of food per dry weight of fish, the percentage daily rations would be: 0.93, 1.03, 1.96, 2.34, and 1.87, respectively.

EFFECTS OF TEMPERATURE ON DEPLETION RATES

Depletion rates increased progressively as acclimation temperatures were increased to 15 C (Fig. 2). Further increase to 19 C did not give further increase in the depletion rate. To examine the effect of acclimation temperature on the length of time for the stomachs to empty, the time to reach 0.01 g was calculated from each regression. An exponential fit does not allow a Y value of zero. A weight of 0.01 g was considered the end point because this was the smallest quantity that could be weighed on the balance used in these experiments. Quantities this small were just barely distinguishable from the thin mucous in the stomach. Figure 2 shows calculations based on experiments repeated within acclimation level and also on the data combined within acclimation level.

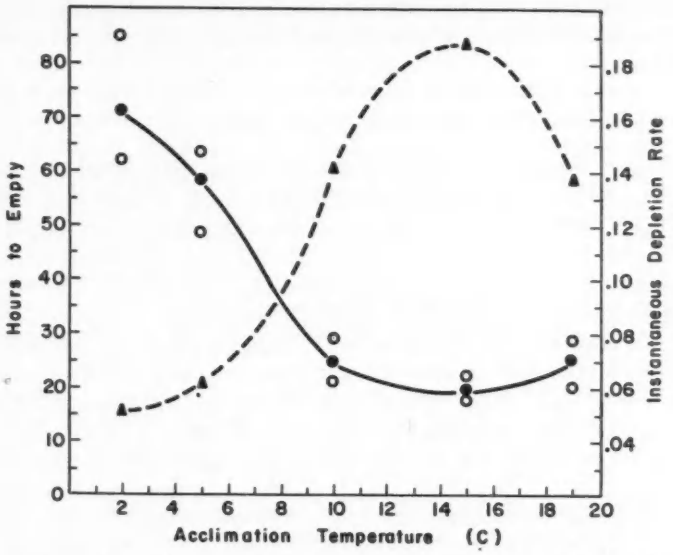

FIG. 2. Relation between time to empty the stomach, rate of depletion, and acclimation temperature. Open circles are calculated from curvilinear regressions on replicate experiments; solid circles are from regressions on combined replicates; triangles are instantaneous depletion rates. Curves are fitted by eye.

Time for complete gastric emptying was 72 hr at 2 C and decreased almost linearly to 58 hr at 5 C and 25 hr at 10 C ($Q_{10} = 3.3$). Further increase in acclimation temperature to 15 and 19 C altered depletion time very little.

Times to 50% depletion were: 13 hr (2 C), 11 hr (5 C), 5 hr (10 C), 4 hr (15 C), 5 hr (19 C).

To find whether regression coefficients (instantaneous depletion rates) at 10 and 19 C were significantly less than the rate at 15 C, one-tailed t-tests were performed. Regression coefficients at 10 and 19 C were both significantly less than the regression coefficient at 15 C ($t = 2.07$, $0.050 > P > 0.025$ for 10–15 C comparison; $t = 2.23$, $0.025 > P > 0.01$ for 15–19 C comparison).

Regression coefficients for data combined within acclimation level are plotted in Fig. 2. Since rate was significantly reduced at 19 C, peak depletion rates possibly lie close to 15 C.

It is also possible, however, that the curve that was fitted to the data is not applicable at the relatively high temperatures of 15 and 19 C. The cod did not feed at 21 C. Gastric emptying mechanisms operating at lower temperatures may be interfered with at 15 and 19 C. It was pointed out earlier that the mean residual from the curvilinear fit to the data given in Fig. 1 was slightly larger than that from the rectilinear fit (0.03 vs. 0.04), and that the residuals of both curvilinear and rectilinear fitting were the same at 15 C. The rectilinear

fit estimates that the stomach empties at 16 hr at 15 C and 15 hr at 19 C. Thus there is argument that there is no difference in emptying rate between 15 and 19 C acclimations.

Rate of Gastric Emptying and Meal Size

Effects of meal size on rate of gastric emptying were tested by two experiments. In the first experiment, fish were acclimated at 5 C and 10 C in separate tanks as previously described and were all given the equivalent of 0.13 g of shrimp dry weight instead of 0.40 g (fish in Fig. 1). Stomachs were opened at intervals after feeding time. Quantities remaining are plotted in Fig. 3 as dry weight and as percentages of initial meal size (dry weight). Points from Fig. 1 (0.40-g meal, dry weight) are included in Fig. 3. At first inspection,

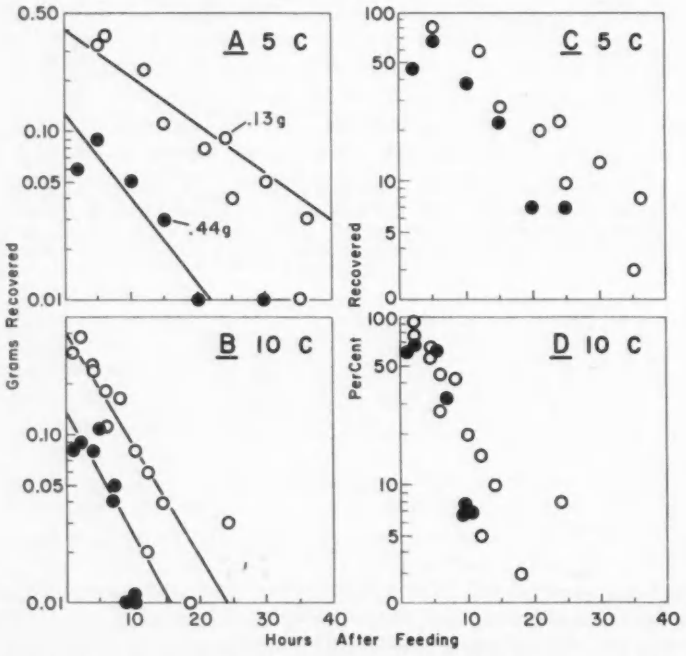

Fig. 3. Depletion of shrimp from stomachs of cod fed two meal sizes (0.13 and 0.44 g dry weight) at two temperature acclimations (5 and 10 C). Each point represents dry weight of shrimp recovered from a fish (panels A and B), or dry weight as a percentage of the initial meal size (panels C and D). Lines in panels A and B are fitted negative exponentials.

the 10 C fitted line in Fig. 3 for the 0.13-g meal does not seem to conform to the points, but it will be noted that the untransformed residuals are about the same at the various times after the meal was given. The line was fitted by curvi-

linear methods on untransformed data rather than a semilog fit. The semilog transformation was used in this figure to facilitate visual comparison. The instantaneous depletion rate at 5 C was −0.11 and at 10 C was −0.16. These were not significantly different from the rates determined with a meal size of 0.40 at the respective temperatures ($t = 0.93$, $P > .05$ at 5 C, and $t = 0.39$, $P > .05$ at 10 C). Thus the effect of changing meal size was simply to shift the stomach emptying curve to a parallel position on the graph. The quantities that had disappeared at various times after the small meal was fed could have been estimated from the large meal experiment by constructing a line through the Y value of 0.13 parallel to the disappearance curve of the large meal in Fig. 3. In other terms, the percentage depletion curves (Fig. 3, panels C and D) for the two meal sizes are statistically indistinguishable. Apparently, the segment of the large meal disappearance curve from 16 to 45 hr at 5 C is equivalent to the small meal curve at 5 C, even though food recovered over this time period had already been in the fishes' stomachs for 16 hr. A similar result follows with the 10 C data. Perhaps digestion occurred only at the surface of the food particle and protein inside the particle was not acted upon until it was exposed.

In the second experiment, tests were performed over a wider range of meal sizes to examine more fully the effect of meal size on rate of gastric emptying. To determine the maximum size meal that a yearling cod would ingest, 10 fish acclimated to 10 C were fed to satiation with shrimp cut as in the experiment already described. The stomachs were immediately opened and the shrimp within the stomach and oesophagus dried to constant weight. The mean quantity consumed was 2.98 g dry weight, standard deviation 0.90 g, range 1.89–4.39 g. This meal was 5.8% of the body weight in terms of dry weight food and fish, or 5.5% on a wet weight basis.

Another series of fish acclimated to 10 and 15 C in separate tanks were fed a range of meal sizes with thawed shrimp prepared and measured as described. All stomachs were opened 6 hr after the meals were consumed. In Fig. 4, meal size for each fish is plotted against quantity remaining in the fish after 6 hr. The greater the meal size, the greater the quantity that remained in the stomach. The solid circles are data given in Fig. 1.

The model used in curve-fitting (equation 2) was considered from the standpoint of the relation in Fig. 4. Equation 2 relates final weight to initial weight as a straight-line function with a zero origin and with slope $= e^{-bt}$. This slope was derived by fitting data in Fig. 4 with a straight line through the origin by least squares regression. Once the quantity e^{-bt} was thus determined empirically (where t, time elapsed since feeding, equals 6 hr), b could be calculated and compared with b values in Table 1 (the regression coefficients of the curvilinear fits in Fig. 1). The calculated b value and 95% confidence interval of 0.12 ± 0.02 at 10 C compares well with $b = 0.14$, derived previously; and at 15 C, the calculated $b = 0.13 \pm 0.05$ compares with $b = 0.18$. Thus, whether one used t or W_0 as an experimental variable, the same b values result. The proposed model seems to be adequate for both experiments.

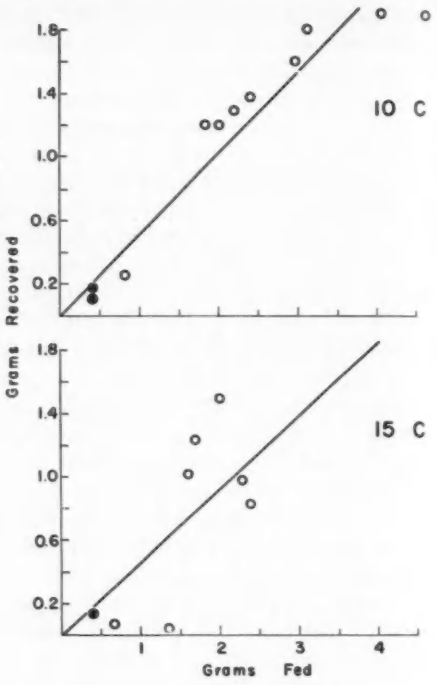

FIG. 4. Relation between weight of shrimp recovered and weight fed at two temperature acclimations. Each point represents one fish except that solid circles are data from Fig. 1. Lines are least square fits with intercepts forced through origin.

MULTIPLE MEAL EXPERIMENT

Experiments reported up to this point were performed on fish with previously cleared stomachs. To compare these results with digestion during which one meal succeeds another before the stomach has time to clear, seven cod acclimated to 5 C were fed three meals at 24-hr intervals and were sampled after the third 24-hr period. The mean meal sizes in order of presentation contained 0.36, 0.28, 0.38 g dry weight. The meals were of wet, thawed shrimp measured as previously described. The expected course of depletion, taken from the 5 C fitted curve in Fig. 1, is plotted in Fig. 5 along with the 95% confidence intervals of the rate, b (Table 1). It was thus estimated that an average of 0.096 g dry weight would remain after 72 hr. After 3 days and three meals, the seven cod had quantities of shrimp in their stomachs ranging from 0.02 to 0.06 g dry weight (Fig. 5) with an average of 0.035 g — a mean outside the 95% confidence interval for the expected value. Possibly, the method of fasting the cod so that they would have empty stomachs at the beginning of the experiments brought about a slight reduction in digestion rates.

Although the discrepancy of 0.061 g dry weight cannot be ignored, it would not be serious enough to prevent estimates of daily ration. The total quantity of food that actually left the stomach during the 3-day period was

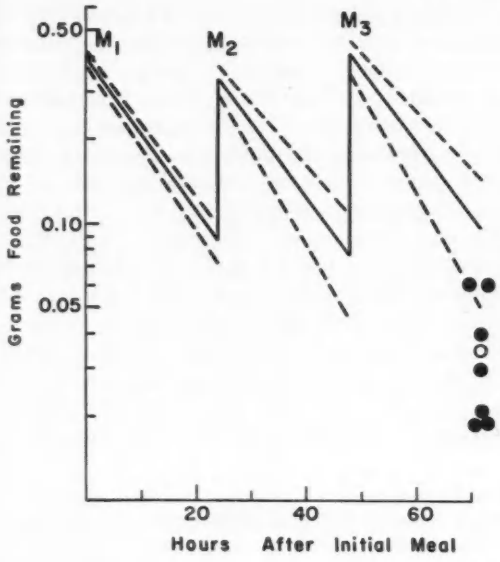

FIG. 5. Weight of shrimp remaining after successive meals at an acclimation temperature of 5 C. Each solid circle represents one fish. The open circle is the mean quantity recovered from the seven fish. The solid line is the predicted course of depletion based on data presented in Fig. 1. Broken lines are calculated from the 95% confidence interval on the fitted slope in Fig. 1. Letters indicate the time at which meals were given.

0.955 g dry weight, or 0.318 g per day. The quantity that was graphically estimated (Fig. 5) to leave the stomach during the period was 0.894 g or 0.298 g per day — a discrepancy of 0.02 g or 7% of the estimated daily ration.

DISCUSSION

EFFECTS OF TEMPERATURE

Instantaneous depletion rate increased rapidly as acclimation temperature increased from 2 to 10 C. Further increase in acclimation temperature altered depletion time little, giving a plateau or shallow hump-shaped response–temperature relation.

Temperature-related depletion rate maxima have not been reported for other fish. Fábián et al. (1963) summarized a series of papers of Molnár and Tölg (1962a, b, c) that showed that log of the time to empty the stomach decreased linearly with acclimation temperature. These authors used largemouth bass (*Micropterus*), pikeperch (*Lucioperca*), catfish (*Silurus*), and perch (*Perca*), and tested between 5 and 25 C. Extension of their data to 30 C might show a plateau region.

Any of four explanations could account for the peak or plateau in the temperature-rate curve. First, activity of a given concentration of proteolytic enzyme could decrease with temperature increase above 15 C. Second, it is possible that progressively less enzyme is formed and available with such temperature increases. Third, the gastric mucosa might not be able to maintain optimum *p*H for enzymatic activity beyond 15 C. Fourth, the rate of gastric

peristalsis could be curtailed with rise in temperature above 15 C. Any or all of these processes could counteract molecular kinetics. The first hypothesis seems unlikely since studies with trout have shown that the gastric proteolytic enzyme, pepsin, increases its activity until 35–40 C (Buchs, 1954). It is possible, however, that temperature acclimation processes influence proteolytic enzyme activity. Regarding the second hypothesis, brown bullheads (*Ictalurus nebulosus*) maintain a constant pepsin concentration at acclimations from 10 to 30 C, although pepsin output by the stomach is maximized at 25 C (Smit, 1967). It is possible that pepsin concentration is rate-limiting to digestion at highest temperature, and hence the plateau in the cod. The third hypothesis is a likely subject for future research. Smit (1967) found that when rate of gastric acid secretion in the bullhead was plotted against acclimation temperature, a hump-shaped curve resulted. This curve was similar in shape to the curve reported in the present study for instantaneous digestion rate versus acclimation temperature, except that the maximum was displaced toward warmer temperatures for the bullhead — a fish that lives in warmer water than does the cod.

There seem to be no published experiments relating amplitude or rate of gastric peristalsis to body (ambient) temperature of poikilothermic vertebrates. Presumably, increased amplitude or rate of gastric motion would bring about more rapid protein hydrolysis through more vigorous mixing of gastric juice with substrate.

Digestion Rate Models

Since the disintegration of a particle being digested probably occurs at the surface of the particle, a model that incorporates particle surface area might be preferable to one that considers only particle weight (volume). As will be shown later, if digestion rate depended on weight alone, the rate of change would be $-b\mathrm{W}$. Since W decreases as a cubic function, the linearization of its change is proportional to the cube root of W. Furthermore, since the surface of the particle decreases as the square of the weight, the rate of change of the particle's weight is proportional to $(\mathrm{W}^{1/3})^2$ or $\mathrm{W}^{2/3}$. Thus, if rate of stomach emptying depended on particle surface area, this rate would be expressed as:

$$\mathrm{dW}/\mathrm{d}t = -a\mathrm{W}^{2/3}. \tag{3}$$

Integrating this expression, one would find that at time t, prior to time for complete digestion, t_F, the weight remaining would be

$$\mathrm{W} = a^3/27\ (t_\mathrm{F} - t)^3 \tag{4}$$

or in linear form

$$\mathrm{W}^{1/3} = kt_\mathrm{F} - kt. \tag{5}$$

The data in this paper are not adequate to eliminate one or the other of the hypothetical volume-dependent or surface-dependent models. It is possible that both volume and surface effects influence rate of emptying. Although it is probable that digestion occurs at the surface of a particle, volume of the food in the stomach might influence rate of peristalsis. The volume-dependent model has advantage in statistical analysis of the data, and therefore has been used in this paper.

Either the surface- or volume-dependent model seems adequate to explain the shape of the gastric emptying plots previously reported for fish, most of which are apparently curvilinear (Kariya, 1969; Kitchell and Windell, 1968; Magnuson, 1969; Pandian, 1967; Pierce, 1936; Windell, 1966; Windell et al., 1969). Gastric emptying rates are also curvilinear in time with humans and both semilog and square-root transformations have been used (Hunt and Knox, 1968). Notable exceptions to this curvilinearity are the depletion plots for sunfish (*Lepomis*) fed mealworms (larva of the beetle, *Tenebrio*), given by Windell (1966) and Kitchell and Windell (1968), which are rectilinear with time and might be better described by equation 1. Also, gastric emptying rates of a piscivorous marine fish, the sea raven (*Hemitripterus americanus*), are rectilinear in time if whole young cod are fed (A. V. Tyler, unpublished data).

Effects of Meal Size

It is instructive to examine the rate expression that can be derived from equation 2. Quantities remaining in the stomach can be estimated by supplying values for parameters in equation 2, but the rate at which depletion occurs must be calculated from the first derivative of this expression

$$dW/dt = -bW_0e^{-bt} \tag{6}$$

substituting from equation 2,

$$dW/dt = -bW. \tag{7}$$

Equation 7 is the slope of the depletion curve fitted in Fig. 1. It was pointed out earlier that this slope, or rate, diminishes as time interval after feeding increases. It can be seen in equation 7 that rate of stomach emptying is dependent on the coefficient b, termed the instantaneous rate of depletion in this paper, and also on the quantity of food in the stomach at any time. The quantity b changes markedly with acclimation temperature, especially between 2 and 10 C. Between 10 and 20 C, other factors being equal, digestion rate is dependent mainly on W.

Effects of quantity of food fed on rate of gastric emptying have been previously studied in sunfish by Windell (1966), Kitchell and Windell (1968), and Windell et al. (1969). As in the present study, these workers found that increased meal sizes resulted in increased depletion rates.

The rate of depletion is proportional to the initial meal size whether the depletion is rectilinear or curvilinear in time. If depletion is curvilinear, the depletion rate is proportional to the mass of food in the stomach at any moment, and for equal time intervals equal percentages of the quantity of food are passed from the stomach. If depletion is rectilinear, a constant quantity is passed per unit of time, and thus an increasing percentage of the diminishing quantity of food.

The maximum rates of depletion that the cod can achieve may be calculated by applying to equation 7 the maximum quantity of food that the fish will hold in its stomach. It has been reported in this paper that this quantity is about 3 g dry weight (12.5 g wet weight) for 229 g fish at 10 C. The value of b at 10 C is 0.14, and so the hourly rate of digestion is 0.42 g dry weight (1.75 g wet weight). If it were possible for the fish to maintain this rate on a 24-hr basis, the fish would pass from its stomach 10.0 g dry weight or 19.7% of its own weight (dry) per day (42.0 g or 18% of its own weight per day on wet weight basis).

Since the rate at which digested food passes out of the stomach is dependent on the quantity of food in the stomach, in young cod metabolic demands may be coupled to intestinal absorption by regulation of the gastric volume. During seasons of highest total metabolism, a cod might continue to feed though it has a "full" stomach, and when metabolic rate is reduced, the fish should maintain some fraction of a full stomach. This postulate, plus the fact of regularity in gastric depletion data, gives cause for optimism regarding estimation of daily rations for fish in their own environment through use of gastric depletion information. One must know the quantities of food in the stomach at different periods of the day and night, and the characteristic depletion rate for specific prey organisms. One must also know whether the swimming activity of the fish influences depletion rate.

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Effects of Gravel Size, Egg Depth, and Egg Density on Intragravel Movement and Emergence of Coho Salmon (*Oncorhynchus kisutch*) Alevins[1]

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Dill, L. M., and T. G. Northcote. 1970. Effects of gravel size, egg depth, and egg density on intragravel movement and emergence of coho salmon (*Oncorhynchus kisutch*) alevins. J. Fish. Res. Bd. Canada 27: 1191–1199.

In experimental aquaria with large gravel (3.2–6.3 cm), vertical and lateral movements of coho salmon (*Oncorhynchus kisutch*) alevins were more extensive and area utilized per alevin was greater than in small gravel (1.9–3.2 cm). At low density (50 per aquarium) the alevins moved farther towards the inlet, but the mean area occupied per alevin was the same as that at high density (100 per aquarium). Burial depths tested (20 and 30 cm) had no significant effect on vertical or lateral movements or on area utilized per alevin. Alevin orientation in the gravel, survival to emergence, and timing of emergence were not affected by any of the environmental variables examined.

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INTRODUCTION

Although the behaviour of trout alevins (*Salmo*, *Salvelinus*) has been extensively studied by White (1915), Stuart (1953), Woodhead (1957), Bishai (1960), Roth and Geiger (1963), and others, only Bams (1969) has examined in detail the behaviour of the alevins of Pacific salmon (*Oncorhynchus*). The studies to date, though often contradictory, suggest that salmonid alevins move about both laterally and vertically within the gravel bed prior to emergence. The present study was designed to examine this possibility in coho salmon (*Oncorhynchus kisutch*) alevins and to investigate effects of gravel size, egg burial depth, and egg density on their intragravel movement and emergence. It has been demonstrated (e.g. Shelton, 1955) that survival and condition at emergence are affected by altering the environment within the redd. Such alterations might also affect the intragravel movements of the alevins. Gravel size and burial depth were chosen because of their significance to design of spawning and incubation channels respectively. Density of planting was varied to examine the possibility of competition between alevins (for example, by feeding before emergence; see Dill, 1967).

MATERIALS AND METHODS

The study was conducted in a laboratory at the University of British Columbia. Eyed eggs of coho salmon (*O. kisutch*) were obtained by excavating a single redd in the Chehalis River,

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B.C., on March 21, 1967. They were held in hatchery troughs (8.3 C), and placed in the 16 experimental aquaria 2 days later.

The aquaria (Fig. 1) measured 66 × 5 × 63 cm high. The base and end frames were made of solid oak and were slotted to receive two panes of 1-cm plexiglass. The area of observation measured 56 × 58 cm. A plastic inlet pipe, 1.3 cm in diameter, was located 25 cm from the bottom

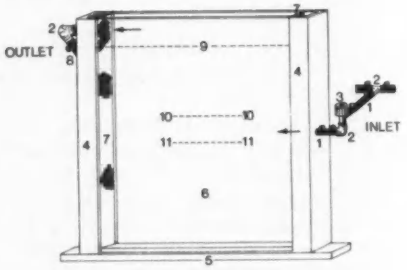

FIG. 1. Schematic diagram of an experimental aquarium. (1) 1.3-cm polyethylene inlet pipe. (2) Plastic fittings on inlet and outlet lines. (3) Nalgene inlet valve. (4) Oak end frame, painted with "Rustoleum" enamel. (5) Oak base painted with "Rustoleum" enamel. (6) 1-cm plexiglass walls inserted into grooves in ends and base and secured with Silastic adhesive. (7) Stainless steel end screens (8 mesh/cm). (8) 2-cm polyethylene outlet pipe. (9) Gravel level. (10) 20-cm egg burial depth. (11) 30-cm egg burial depth.

on one end and a 2-cm plastic pipe served as the outlet on the other end of each aquarium, 2.5 cm from the top. Gravel was retained between two stainless steel screens, located in slots at each end. Water flow was controlled with a Nalgene spigot valve on each inlet. The water was dechlorinated and all piping and fittings were plastic.

Gravel size, egg burial depth, and egg density were each tested at two levels, providing a 2^3 factorial design. The egg densities were 50 and 100 eggs per aquarium. The burial depths were 20 and 30 cm. Large and small gravel sizes were 3.2–6.3 and 1.9–3.2 cm respectively. Forty-six cm of gravel were placed in each aquarium on March 18, 1967. Five days later the eggs were deposited through plastic pipes, usually in a single small pocket near the front plexiglass plate. The pipes were removed thereafter.

The tanks were lighted from 40 cm above the gravel surface by 40-w "cool white" fluorescent tubes connected to an electric timing device adjusted to the natural day–night rhythm (but without dusk and dawn periods). Light was largely prevented from striking the fronts or backs of the aquaria by means of black polyethylene curtains. Some light may have been able to reach alevins against the plate because of the transmittant properties of the plexiglass. The entire area was surrounded by additional curtains of black polyethylene to minimize extraneous stimuli.

Mean daily water temperatures ranged between 6.5 and 10.9 C over the experimental period of 2½ months. Variation between aquaria did not exceed 0.5 C. Water samples for dissolved oxygen were taken June 16 from the outlet ends of four large and four small gravel aquaria and analyzed by unmodified Winkler method. The mean oxygen concentration of the water in the small gravel aquaria was 12.3 mg/liter (range 12.1–12.4 mg), and in the large gravel aquaria was 12.1 mg/liter (range 11.8–12.6 mg). Oxygen and temperature conditions, therefore, were considered to have been similar in all treatments at least on a macroscale. Microscale differences existed, however, as evidenced by 100% mortality of eggs in one aquarium in which deflection of water flow in the gravel apparently caused an oxygen shortage in the immediate vicinity of the egg pocket.

Intragravel flow was measured once between June 13–16 in five aquaria, three containing small gravel and two containing large gravel. Approximately 1 ml of Rhodamine B dye was injected into the inlet pipe of each aquarium above the inlet control valve, and its distribution in the gravel recorded at 2-min intervals to determine flow pattern and rate. Flow pattern in both gravel sizes was horizontal in the middle of the aquaria, but tended to upwell at the edges. Flow rates through the gravel were approximately 2.4 and 6.2 cm/min in the small and large gravels respectively.

Daytime observations were begun as soon as hatching started. With but a few exceptions, these were made every day from April 14 to May 18, and every 2nd day from May 18 to June 10 in the large gravel and from May 18 to July 10 in the small gravel. The position and orientation of each visible alevin was noted by placing a grid (2.5 × 2.5 cm squares) on the front face of the

aquarium and recording on squared paper. Lifting the black plastic curtain of each aquarium allowed enough light to carry out this operation (usually less than 5 min duration).

Some night observations were made with an infrared viewer on April 18 and 29, May 14, 19, 29, and 30, June 1, and July 9. The infrared light source consisted of two 6-v lamps covered by Kodak #87 Wratten filters. Resolution was imperfect and it was often difficult to locate all of the alevins known to be in the gravel. For this reason, results of nighttime observations were neither as complete nor as accurate as their daytime counterparts.

Mean lateral and vertical positions of alevins in the gravel bed were determined for each observation date by assigning a number to each grid section, multiplying this number by the number of fish in the section, summing over all sections, and dividing this value by the total number of fish visible.

The amount of downward movement was expressed as a percentage of the distance from the position of burial to the bottom of the aquarium. The amount of upward movement was calculated as a percentage of the distance from the lowest mean depth recorded to the surface of the gravel. Lateral movement was quantified by maximum extent of movement towards inlet and outlet ends of the aquaria.

The directions assumed by alevins whose longitudinal axes were approximately parallel to the sides of the aquaria at the time of observation were recorded. Orientation around the horizontal frontocaudal axis (roll) was not considered. For purposes of analysis the angles (α) recorded were reduced to only four, counterclockwise from top center: 90° (representing all fish orienting between 45 and 135°), 180° (135 to 225°), 270° (225 to 315°), and 360° (315 to 45°). The following set of equations was used to determine from tables the vector resultant (θ) for the empirical circular distribution (Batschelet, 1965):

$$x = \frac{1}{n}(\cos \alpha_1 + \cos \alpha_2 + \ldots \cos \alpha_n) = \frac{1}{n}\sum_{i=1}^{n} \cos \alpha_i$$

$$y = \frac{1}{n}(\sin \alpha_1 + \sin \alpha_2 + \ldots \sin \alpha_n) = \frac{1}{n}\sum_{i=1}^{n} \sin \alpha_i$$

$$r = \sqrt{x^2 + y^2}$$

$$\cos \theta = x/r$$

$$\sin \theta = y/r$$

The mean angles of orientation in the aquaria were determined for the following periods: first hatch–April 30, May 1–10, May 11–20, May 21–31, and June 1–end of emergence (June 10 in the large gravel; July 10 in the small gravel). The data were also averaged over the entire period in which alevins or fry were observed in the gravel.

When three or more alevins were present in the observation area their positions were recorded and connected by straight lines. The area contained within these lines was measured with a planimeter and divided by the total number of alevins observed to determine the mean area occupied per alevin. Data were tabulated for each observation day for each aquarium but only maximum area occupied per alevin was statistically analyzed.

The percent survival was determined by dividing the number of fry captured by the number of eggs planted. Survival to emergence data are therefore minimal, since some fish may have been lost from the system, although screens in the tanks made this unlikely. At the end of the experiment, organic remains in the aquaria were examined but were usually in such an advanced state of decay as to be beyond enumeration.

Days after planting to the first emerged fry observed was the only parameter used to compare emergence timing between treatments. Days to maximum and last emergence were impossible to determine in the large gravel due to the ease of reentry by the fry.

Differences between treatments were analyzed by the Yates tabular method of factorial analysis (Davies, 1956) at $P = 0.05$. Replicates of four treatments were lost. The eggs in one died because of a water system failure, and those in another probably died because of low oxygen levels (see above). Two others provided data only on emergence timing since the eggs were inadvertently buried in the middle of the tank and few alevins could be seen. No attempt was made to estimate the values of these lost replicates. If both replicates of a treatment were available, they were averaged to provide the data used in the analysis.

RESULTS

General description of behaviour — Within a day after hatching, the alevins moved downward. This downward phase was more marked in the large than in the small gravel, possibly because of greater ease of movement in the former. In large gravel the alevins, after reaching the bottom of the tank, grouped closely together but occasionally scattered rapidly when one member of the aggregation moved. The group of alevins would subsequently reform. Infrared observations indicated that the fry were dispersed farther at night than during the day. With progressive absorption of their yolk sacs the alevins began to spread out along the bottoms of the aquaria. From there they made temporary excursions into the upper regions of the gravel (10 cm or less from the bottom) and finally emerged.

In the small gravel, alevins reached the bottom in only two aquaria. Downward movement was slight in most instances and was associated with dispersal in all directions from the point of hatching. Most alevins moved upward and towards the ends of the aquaria during emergence, although some did move straight up. This pattern, extended three-dimensionally beyond the width confines of the aquaria, would approximate a solid cone, with the apex slightly below the point of hatching.

Considerable daytime emergence was observed in small gravel aquaria where the fry, although able to emerge from the tightly packed substrate, apparently were not able to force their way back into it after emergence. Daytime emergence from the large gravel may also have occurred but could not be detected because of the ease with which fry could reenter the gravel. Hourly electronic flash photographs in one small gravel aquarium (8:00 PM June 7 to 9:00 AM June 8) showed a slow but continuous upward movement throughout the night, with most fry emerging near morning (6:30 AM).

Extent of intragravel movement — Alevins moved significantly farther during the downward phase in the large gravel than in the small gravel (Table 1 and Fig. 2)[2]. Egg depth and density had no significant effect. There was no effect on extent of upward movement of any of the environmental factors studied.

[2]Variances for the parameter values shown in Fig. 2–4 are great on any given day since the various treatments were often out of phase with one another. For example, in Fig. 2, alevins in some treatments had reached the bottom of the aquaria whereas those in others were just beginning to move downward.

TABLE 1. The percent vertical movement[a] by coho alevins in the experimental aquaria in relation to gravel size, egg burial depth, and density.

Egg density (*no./aquarium*)	Direction	Burial depth in large gravel		Burial depth in small gravel	
		20 cm	30 cm	20 cm	30 cm
50	Down	100.0	100.0	37.0	28.1
	Up	90.0	64.0	80.0	64.0
100	Down	100.0	100.0	19.0	17.7
	Up	69.0	83.0	63.0	47.0

[a]See methods for explanation.

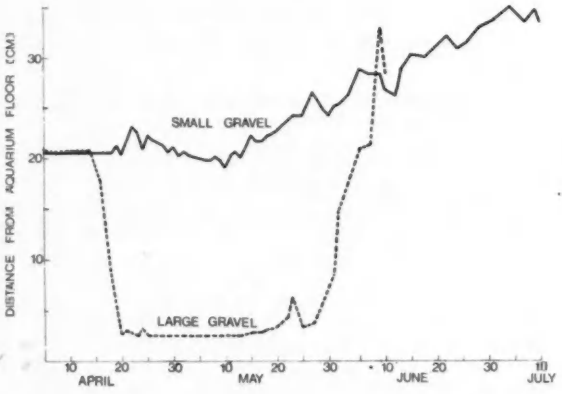

FIG. 2. Generalized pattern of changes in vertical distribution of coho alevins in large and small gravel aquaria (data averaged over all combinations of egg burial depth and egg density).

Lateral movement (Table 2 and Fig. 3) during the downward phase was directed with the current and was significantly greater in the large than in the small gravel. The lateral movement during the upward phase of the migration was predominantly directed into the current and was significantly greater at low than at high egg density. The other environmental factors studied had no significant effects.

TABLE 2. Lateral movements of coho alevins (expressed as cm from aquarium centre) in relation to gravel size, egg burial depth, and density.

Egg density (*no./aquarium*)	Direction	Burial depth in large gravel		Burial depth in small gravel	
		20 cm	30 cm	20 cm	30 cm
50	With current	16.0	15.7	0.5	3.8
	Into current	20.8	21.1	15.7	11.9
100	With current	14.0	19.8	6.4	4.6
	Into current	7.4	13.0	9.7	7.1

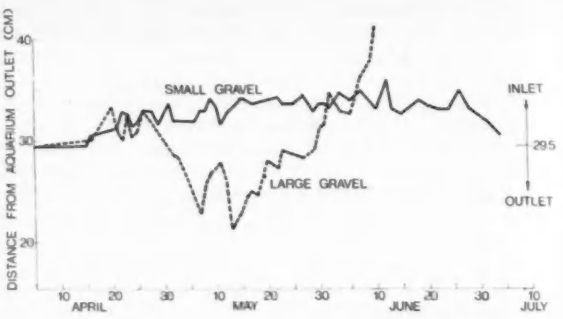

FIG. 3. Generalized pattern of changes in lateral distribution of coho alevins in large and small gravel aquaria (data averaged over all combinations of egg burial depth and egg density).

Orientation in the gravel — The most common direction of alevin orientation (Table 3) lay between 270 and 360°, i.e., upwards and into the current. Orientation upwards and with the current was also occasionally recorded, especially

TABLE 3. Sequential changes in mean orientation directions (degrees, counterclockwise from top center) of coho alevins in the experimental aquaria.

Treatment		Date					
Burial depth	Egg density	Hatch–Apr. 30	May 1–10	May 11–20	May 21–31	June 1–end	Hatch–end
			Small gravel				
20 cm	50	337	333	343	345	356	348
	100	46	360	347	343	358	357
30 cm	50	214	284	328	342	354	339
	100	135	338	351	213	270	310
			Large gravel				
20 cm	50	5	8	272	277	342	325
	100	270	90	50	303	352	330
30 cm	50	360	88	315	330	334	349
	100	352	27	323	347	357	33

in the large gravel aquaria. Downward orientation was noted in only a few instances, all in the small gravel. Angle of orientation, averaged over the entire period in which alevins were observed in the gravel (Table 3, "Hatch–end"), was not significantly affected by gravel size, egg depth, or density.

Spatial distribution in the gravel — The average area occupied by each alevin increased with time (Fig. 4). The maximum average area occupied (Table 4) was significantly greater in large than in small gravel aquaria. Average area occupied per alevin was not significantly affected by burial depth or egg density. However, the total area occupied was greater at the higher density, i.e., regardless of density each alevin occupied about the same gravel area.

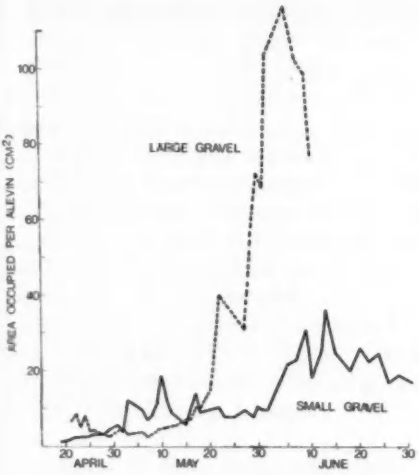

FIG. 4. Generalized pattern of changes in spatial distribution of coho alevins in large and small gravel aquaria (data averaged over all combinations of egg burial depth and egg density).

TABLE 4. Maximum area utilized per alevin (square cm per visible alevin) in the experimental aquaria in relation to gravel size, egg burial depth, and density.

Egg density (*no./ aquarium*)	Burial depth in large gravel		Burial depth in small gravel	
	20 cm	30 cm	20 cm	30 cm
50	181.0	176.6	55.7	71.9
100	198.6	183.4	41.3	20.8

Emergence — Survival to the fry stage and time from deposition to first emergence was not significantly affected by gravel size, egg burial depth, or egg density (Table 5). Small gravel greatly lengthened the period of emergence,

TABLE 5. Egg-to-fry survival and emergence timing of coho alevins in the experimental aquaria in relation to gravel size, egg burial depth, and density.

Egg density (*no./ aquarium*)	Burial depth in large gravel		Burial depth in small gravel	
	20 cm	30 cm	20 cm	30 cm
	% Egg-to-fry survival			
50	94	92	46	45
100	52	86	76	30
	Days after planting to first emergence			
50	67	71	67	68
100	67	67	67	73

however, since some fry were still emerging 1 month after emergence had been completed in the large gravel.

DISCUSSION

Two considerations must be borne in mind throughout discussion of these results. First, behaviour of the alevins near or at the plexiglass front plates was assumed to be similar to that of alevins located farther back in the gravel even though the former may have been exposed to more light as a result of the light-transmitting property of the plexiglass and the method of observation. However, in two aquaria where all eggs were inadvertently buried away from the front plexiglass plate, timing of alevin emergence was similar to that in the other replicates, where eggs were placed adjacent to the plate. This suggested a minimal effect of extraneous light, at least on alevin emergence. Secondly, all eggs were obtained from a single redd (probably from a single female) and no doubt exhibited less genetic variability than might be expected from a broader parental origin.

The coho alevins did move about within the gravel prior to emergence and this movement had both lateral and vertical components. Roth and Geiger (1963) demonstrated that the intragravel movements of brown trout (*Salmo trutta*) have a pronounced vertical component, the alevins moving downward prior to their upward migration to the gravel surface. Stuart (1953) suggested that intragravel movements in brown trout have an important lateral component as well, which Roth was unable to observe in his cylindrical experimental observation chambers, since these allowed little lateral movement. Stuart stated that "migration . . . was in a lateral direction and never directly upwards. The alevins dispersed into the gravel . . . radiating upwards and outwards, and a plot of their relative positions took the form of an inverted cone." A similar pattern of movement has been noted for coho salmon and steelhead trout (*S. gairdneri*) alevins in Oregon (H. J. Campbell, personal communication) and was also observed in the present study. The lateral and vertical movements and average area occupied by the coho alevins were greater in large than in small gravel aquaria possibly because of the combined effects of less restricted movement and greater light penetration in the larger substrate.

High egg density decreased the amount of lateral movement of alevins towards the inlet (into the current) during the upward phase, but had no apparent effect on the area occupied per alevin. The latter observation could be interpreted as evidence of competition in the gravel either for oxygen or for food, at least in the later stages.

Alevins in large gravel, where light probably penetrated the farthest, showed more downward movement than did alevins in small gravel. The alevins in the large gravel also were more active at night than in the day. Both observations suggest that coho salmon alevins are negatively phototactic until they begin their upward movement and are either positively phototactic or neutral to light thereafter. Such a change in phototaxis with development has been suggested by Stuart (1953) and Roth and Geiger (1963) in brown trout. In

addition White (1915) and Woodhead (1957) have shown that newly hatched *Salmo* and *Salvelinus* larvae are negatively phototactic. Bams (1969), on the other hand, found that sockeye (*Oncorhynchus nerka*) alevins were negatively phototactic in the final stages of their intragravel existence, and that any light inhibited emergence.

The coho alevins in the present study were positively rheotactic in later stages, but apparently negatively rheotactic during the downward phase, as both Bishai (1960) and Stuart (1953) observed for brown trout alevins. White (1915) found that brook trout alevins were positively rheotactic at the time of hatching, and Roth and Geiger (1963) reported that brown trout alevins were positively rheotactic during the entire alevin stage. Bams (1969) showed that sockeye and pink salmon (*O. gorbuscha*) alevins were positively rheotactic in the presence of light. Further studies are needed to determine whether the reported differences are species-specific or due to the variety of experimental techniques used.

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Flavor Effects of Different Woods on Whitefish Smoked in a Kiln with Controlled Temperature, Humidity, and Air Velocity

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LANTZ, A. W., AND M. VAISEY. 1970. Flavor effects of different woods on whitefish smoked in a kiln with controlled temperature, humidity, and air velocity. J. Fish. Res. Bd. Canada 27: 1201–1207.

The use of a stainless steel kiln capable of careful environmental control permitted a comparison of the effects of 12 different Canadian woods on the flavor of smoked whitefish (*Coregonus clupeaformis*). A panel of 61 untrained judges, scoring on a 9-point hedonic scale, liked canned smoked whitefish better when it was smoked with red maple, red oak, trembling aspen, white ash, balsam, and birch. The flavors from beech and white oak were moderately well liked, whereas those from hard maple and hickory were of only borderline acceptability. Diamond willow and burr oak gave distinctly distasteful flavors. Comments from the untrained judges together with flavor descriptions from a panel of five trained judges classified distasteful flavors as medicinal, astringent, gasolinelike, and sour, depending on the wood source. Smoked products from female fish were liked slightly better than those from male fish.

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INTRODUCTION

IN THE PRESENT STUDY the acceptability of canned whitefish (*Coregonus clupeaformis*) presmoked with 12 different woods was examined. Such knowledge could help the fish processor to select wood smoke sources that would give maximum consumer acceptance to his product.

During earlier investigations of canned smoked fish fillets at the Freshwater Institute, flavors with undesirable characteristics were occasionally detected although not identified. As the fish quality was fairly constant in these studies, the smoke source was considered a possible cause of flavor variability. However, the sawdust used was a mixture of hard and soft woods supplied by local sawmills, hence the wood source was impossible to identify, even by microscopy (Cutting, 1965).

Only a modest amount of work has been reported on the flavor of foods smoked with different woods. However, the advertising of both maple- and

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hickory-smoked bacon indicates that flavors can be unique for different smoke sources. Spanyar et al. (1960) have suggested that the smoke volatiles absorbed by a food may vary according to both the nature of the food and the smoke source. In general, food processors have confined their smoke sources to hardwoods. In an examination of a variety of British Columbia soft wood sawdusts, Sidaway (1944) found that fir and red cedar imparted a resinous flavor to smoked herring (*Clupea harengus pallasi*). Tilgner and Wierzbicka (1959) in Poland reported that hot-smoked herring proved best when smoked with beech, oak, or maple; pine and birch gave almost as good products, but alder, lime, aspen, and fir all gave inferior products. These same workers found that sprats (*Clupea sprattus*) smoked with pine were superior to those smoked with beech. The woods used in the present study included some of the soft and hard woods widely distributed throughout the wooded areas of the Canadian Prairie Provinces and Ontario.

EXPERIMENTAL METHOD

The flavors imparted by smoke from 12 different woods (Table 1) were examined in canned presmoked whitefish by both an untrained 61-member panel and a trained five-member discussion panel.

TABLE 1. Identities and moisture contents of different woods used as smoke sources.

Common name	Botanical name[a]	Wood chip moisture (%)
Ash, white	*Fraxinus americana* L.	20.7
Aspen, trembling	*Populus tremuloides* Michx.	32.7
Beech	*Fagus grandifolia* Ehrh.	26.3
Birch	*Betula papyrifera* Marsh.	21.0
Hickory	*Carya*	24.3
Maple, hard	*Acer saccharum* Marsh.	33.7
Maple, red	*Acer rubrum* L.	30.0
Oak, burr	*Quercus macrocarpa* Michx.	10.5
Oak, red	*Quercus rubra* L.	32.3
Oak, white	*Quercus alba* L.	28.7
Poplar, balsam	*Populus balsamifera* L.	42.0
Willow, diamond	*Salix cordata*	17.5

[a]Identification of different woods as to common and botanical names through the courtesy of Department of Northern Affairs and Natural Resources, Forestry Branch, Ottawa, Ont.

FISH HANDLING

Experimental fish were obtained from a single catch at Lake Winnipeg in March 1969, were well iced, and brought directly to the Freshwater Institute in styrofoam boxes. All were then immediately separated into male and female lots, sorted by weight to exclude those outside of the 500–840-g range, filleted, and assigned at random to the wood smoke treatments. In all, 24 fillets were assigned to each smoke treatment, 3 lots each of male and female fish with each lot consisting of 4 fillets. Each lot was vacuum-sealed in a polyethylene bag, frozen in a plate freezer, and held at −37 C until processed within the following 4 weeks.

For processing, fillets were thawed, smoked at 90 F (32 C) for 45 min without brining, cut into 1½-inch (3.8-cm) steaks, and packed in ¼-lb (113-g) cans. A single salt pellet (1.3 g NaCl) was added to each can. Cans were then autoclaved at 116 C for 60 min. To allow for salt penetration throughout the flesh, all cans were held at room temperature for 1 month before sensory testing.

SMOKING

For all wood species, logs approximately 4–5 ft (122–152 cm) in length and 5–6 inches (12–15 cm) in diameter were obtained and debarked just prior to chipping. The Ergo wood chipper[1] was used to cut the logs into ¼-inch (.64-cm) chips suitable for feeding into the hopper of a Mo-Tra-Fa smoke generator[2] (Fig. 1). Details of smoke generation have been described by Lantz (1966).

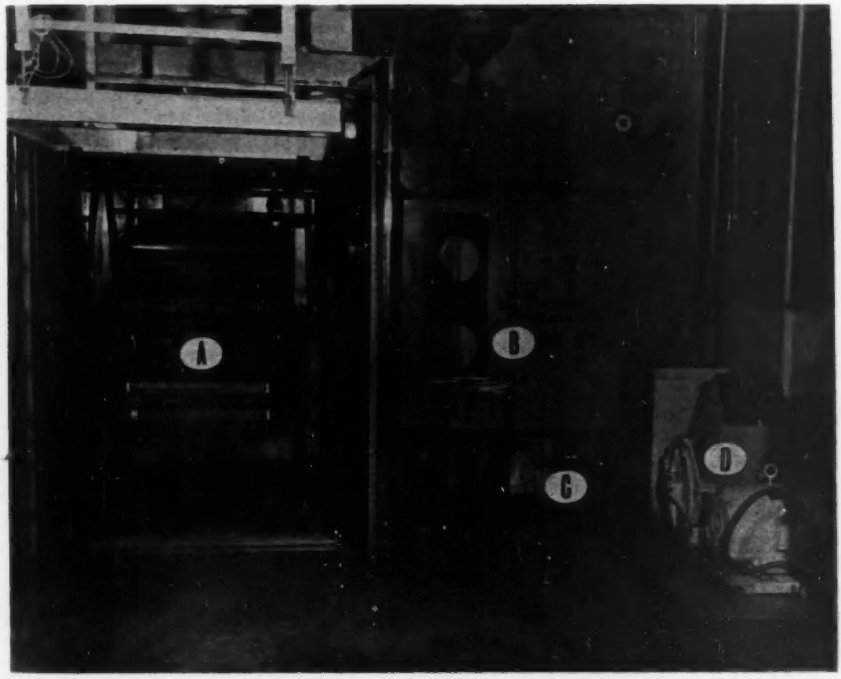

FIG. 1. Freshwater Institute smoke kiln. (A) Doors opened to expose the rotating baskets and wheel conveyor. (B) Control panel. (C) Behind the control panel Mo-Tra-Fa smoke generator. Note that the base of smoke generator is visible below the control panel. (D) Extreme right, Ergo wood chipper and box container for holding wood chips.

All fish lots assigned to each wood were smoked at the same time for 95 min at 32 C and 55% relative humidity in revolving stainless steel baskets mounted on a wheel frame located in a stainless steel kiln. The basket rotation system has the advantage of exposing the fish uniformly to smoke (Lantz, 1966). The stainless steel kiln[3] further controls the smoking operation so that experimental comparisons of the process can be made with greater confidence. The kiln (Fig. 1) is equipped with automatic temperature and humidity controls along with fans for control of air volume from 960 to 2460 ft³/min. Programming instruments permit control of dry- and wet-bulb

[1]Manufactured by TRA OCH BYGGE, Ljungby, Sweden.
[2]Built by Paul Wiencke, Jr., Oslo, Norway.
[3]Designed by Alkar Rasmussen Engineering Ltd., Box 196, Lodi, Wisconsin, USA.

temperatures within a 2-degree C differential. Preselected smoking and drying cycles can be operated and controlled automatically from the pneumatic instrument panel. Fresh air is conditioned by refrigeration as low as 15 C. Temperature and humidity are recorded continuously during the processing. This kiln is equipped with an automatic cleaning device for washdown and removal of smoke constituents. This feature permitted thorough cleaning between wood treatments.

Sensory Evaluation

Sixty-one university students and employees tasted both fish sexes smoked with each of the 12 wood smokes. Treatments were arranged as a three-way factorial experiment. Every panelist judged all 24 treatments by examining 8 at each of 3 sittings held over 3 consecutive days. The serving order of treatments was randomized over all sittings for each panelist. Sessions were held in a well-lit, air-conditioned room with individual judging locations.

Samples of whitefish were prepared by draining the cans and fork-flaking together fish from one treatment to enhance homogeneity. Samples weighing approximately 15 g were placed in coded plastic cups with cardboard lids. Samples were at room temperature when served. Before each sitting, each subject was given a sample from a slurry of smoked fish treatments to provide familiarity with this product and to establish a uniform degree of sensory adaptation. Unsalted soda crackers were supplied as accompaniments and milk was provided as the rinsing medium. Rinsing after each treatment was recommended.

As these panelists were untrained in judging smoked fish, the questionnaire required only a simple hedonic response for each treatment on a 9-point scale (Amerine et al., 1965). In addition, the panelists were requested to select appropriate criticisms of the flavor from the following list: too salty, too smoky, too bitter, too bland, too sweet, medicinal, burnt, fishy-rancid, no comment. Analysis of variance was applied to the numerical values later assigned to the hedonic scale points used.

To describe the flavors in more detail, a panel of five judges trained in tasting smoked fish met to examine all samples. After individual recording of flavor impressions for each treatment, informal discussion was conducted to yield a consensus.

Chemical Analyses

The moisture content of fresh wood chips from each wood species was determined (Association of Official Agricultural Chemists, 1965, p. 346). Percent salt of the canned fish was measured as sodium using atomic absorption spectrophotometry.

RESULTS

The smoke source clearly affected the acceptability of canned smoked whitefish (Table 2). Of the 12 woods examined, results from the untrained panel showed that significantly better-liked flavors were produced by red maple, red oak, trembling aspen, white ash, balsam poplar, and birch. Beech and white oak were moderately acceptable smoke sources, whereas diamond willow and burr oak produced uniquely distasteful flavors. Hard maple and hickory seemed of only borderline acceptability. Adverse criticisms were more frequent for the least-liked products. Flavor descriptions by the trained panel were more favorable for the woods that were better-liked by the untrained panel. The top-scoring wood had a desirable blend of salty, sweet, and smoky flavor notes that were recognized quickly on tasting. Less well-liked products had flavors that were less rapidly apparent and included sour, burnt, bitter, medicinal, or gasolinelike notes.

The more acceptable fish products were criticized as being too bland approximately 20% of the time. At the conclusion of this study several of the

TABLE 2. Acceptability of whitefish smoked with different woods.

Wood	Untrained panel judgments: Mean[a] hedonic score (max = 9)	Untrained panel judgments: Adverse criticisms mentioned > 12 times	Trained panel flavor comments
Red maple	6.49[b]	–	Good, "tight" blend of salty, sweet, smoky
Red oak	6.42[b]	–	Good; slight sour and burnt notes
Trembling aspen	6.24[b]	–	Good; slower flavor impact; sweet
White ash	6.08[b]	Too smoky	Good; slight medicinal taste
Balsam poplar	6.05[b]	–	Slower impact; sweet, mild
Birch	5.98[b]	–	Good blend of sweet and smoky; medicinal
Beech	5.91[b,c]	–	Good; flat taste; slightly burnt
White oak	5.82[c,d]	–	Fair; slight medicinal bitter taste
Hard maple	5.32[d,e]	Too smoky, too fishy	Dominant sweet–sour taste; fishy
Hickory	5.24[e,f]	Too smoky, medicinal	Salt and smoky flavors poorly blended
Diamond willow	4.70[f]	Medicinal	Strong, fruity, sweet, medicinal, astringent
Burr oak	3.77[g]	Too smoky, medicinal fishy	Unacceptable; strong medicinal, gasoline-like taste

[a]N = 122: 61 judges × 2 fish sexes.
[b–g]Values not bearing the same alphabetical letter are significantly different at $P < 0.01$.

judges voluntarily expressed willingness to taste smoked fish again; that is, generally they liked this product.

The smoked products from female fish were better liked generally by the untrained panelists than those from male fish (Table 3). However, adverse

TABLE 3. Flavor preferences according to sex of canned smoked whitefish.

Wood	Consumer panel hedonic score (max = 9)		Salt content (%)	
	Male	Female	Male	Female
Red maple	6.41	6.57	1.49	1.51
Red oak	6.29	6.57	1.29	1.29
Trembling aspen	6.13	6.34	1.08	0.73
White ash	5.95	6.20	1.29	1.61
Balsam poplar	6.05	6.05	1.05	1.45
Birch	5.90	6.05	1.30	1.69
Beech	5.77	6.05	1.43	0.92
White oak	5.90	5.75	1.40	1.29
Hard maple	5.08	5.57	1.32	1.43
Hickory	4.97	5.52	1.29	0.95
Diamond willow	4.64	4.75	1.37	0.94
Burr oak	3.77	3.77	1.24	1.14
Mean	5.71[a]	5.78[a]	1.30	1.25

[a]Mean values show female fish scored significantly higher ($P < 0.05$).

criticisms from the untrained panel showed no relation to fish sex, nor did the observations of the trained panel. Salt contents on the average were not different.

Reasons for the flavor differences dependent on wood sources must await a comparison of the chemical characteristics of the smoke volatiles. Moisture values of the wood chips (Table 1) compared favorably with those for hard and soft wood sawdusts reported by Cutting (1965), with the exception of burr oak. Nevertheless, Spanyar et al. (1960) reported that wood moisture did not affect the composition of smoke volatiles. All treatments had salt levels within the acceptable range of 1–2% (Table 3).

Analytical work on smoke from various woods has shown that the volatiles consist largely of phenols, carbonyls, and acids (Porter et al., 1965). Bratzler et al. (1969) believe that, of these three groups of compounds, the phenols are the most significant to smoked flavor. Using laboratory-prepared samples of bologna, they showed that carbonyls and acids played only a minor role in the total smoked flavor experience. Accordingly, Bratzler and Robinson (1967) have used total phenols as an estimate of the smoked flavor intensity of chub.

The differences in smoked flavor between the various woods examined in this study suggest that total phenols would provide a valid estimate of smoked flavor intensity only when the same wood source is used. It is highly likely that different woods possess phenolic compounds that differ both in type and proportion. Wasserman (1966) has shown that guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol, all isolated from hickory smoke, varied in the intensities of the smoky flavor they produced. Also each generated some sweet, bitter, and phenolic or medicinal tastes.

A sweet taste was observed in several of the treatments studied (Table 2). W. Fiddler et al. (unpublished data) have suggested that sweet tastes may be attributable to carmelization products or to the presence of vanillin; this and other carbonyl compounds may significantly mask phenolic or medicinal tastes.

Although the present work did not include chemical examination of smoke volatiles, the distinctive flavors observed in the same product smoked with different woods certainly suggest different chemical compositions. Clearly some woods are more acceptable sources than others for smoking whitefish.

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Influence of Temperature and Salinity Acclimation on Temperature Preferenda of the Euryhaline Fish *Tilapia nilotica*

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When *Tilapia nilotica* was acclimated to temperatures of 15–35 C and salinities of 0–30‰ in a vertical gradient tank, maximum temperature preferenda occurred at acclimation temperatures of 20 and 25 irrespective of salinity. Preferenda declined as acclimation temperature was increased above 25 C and, except at 0 and 7.5‰, declined as acclimation temperature was decreased below 20 C. The pattern of the relation between final temperature preferenda and salinity was similar to that reported between oxygen consumption for a given sustained swimming speed and salinity. The final preferendum was lowest at 15‰, close to the isosmotic salinity of *T. nilotica*, and highest at the extremes, 0 and 30‰. Final temperature preferenda are in general agreement with optimum temperatures reported for growth, reproduction, and swimming performance.

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INTRODUCTION

THIS PAPER reports on the temperature preferenda of *Tilapia nilotica* in relation to acclimation temperature and salinity. Species of the genus *Tilapia* are particularly tolerant of salinity (Spector, 1956). The thermal range of *T. nilotica* extends from about 7 C (Avault and Shell, 1966) to 42 C (Denzer, 1966). Information on the combined influence of salinity and temperature acclimation on the temperature preferendum is not available for this fish.

Fish exposed to a broad thermal gradient usually congregate within a narrow range of the temperatures available. This behavioural response to temperature has been variously termed preferred temperature, temperature preferendum, and selected temperature. In this report, the term temperature preferendum (Fry, 1964) is followed.

MATERIALS AND METHODS

Tilapia nilotica were obtained from a stock maintained in our laboratory at the University of Guelph.

Fish were held in 75-liter holding tanks and fed to satiation once daily on a commercial food in pellet form (J. R. Clark Co., Salt Lake City, Utah). A photoperiod of approximately 15 hr was maintained throughout the study. Prophylactic treatment was not used since disease was not apparent during the study.

In acclimating fish to different temperatures, a daily change of 1 degree C was followed. After the desired temperature was reached a further period of at least 4 days was allowed. At each acclimation temperature, fish were acclimated to fresh water (0‰) and to salinities of 7.5, 15.0, 22.5, and 30‰. Salinity was altered by 1‰/day by the addition of artificial marine salt (New Tropic-Marin, U. K. Shirley Aquatics Ltd.) until the desired salinity was reached. Fish

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were allowed an additional 4 days at the final salinity before use in an experiment. Salinities were checked daily with an induction salinometer and occasionally by the high precision method outlined in Strickland and Parsons (1968).

Experiments were performed in a tank similar to that described by Brett (1952) except that the entire tank was constructed of acrylic. Outside dimensions were, vertical, 90 cm; horizontal, 60 × 45 cm. To establish and maintain a temperature gradient, two rectangular coils of aluminum tubing each of seven turns were suspended in the experimental tank. Warm water was passed through one coil, entering at the top of the tank and leaving at the bottom. Cold water was passed through the second coil in the opposite direction. The side of the tank designated as the front was marked with horizontal lines to form 12 zones each 7.5 cm high. Temperature was measured by 12 thermistor probes evenly spaced along one side of the tank so that each thermistor recorded from the approximate vertical midpoint of a zone. Overhead incandescent lights provided a light intensity of about 60 lux at the surface of the experimental tank.

Temperature and salinity were adjusted to correspond to acclimation conditions before the introduction of 10 fish, placed in the experimental tank 16 hr before each experiment. Aeration before an experiment assisted in maintaining oxygen concentrations above 80% of air saturation. Carbon dioxide remained below 4 mg/liter and ammonia nitrogen less than 0.1 mg/liter. Methods of chemical analyses followed were those recommended by the American Public Health Association (1960). Fish were not fed for 24 hr before an experiment.

An experiment was begun by removing the aerator, and, after a 30-min period but before the establishment of a thermal gradient, distribution of fish was recorded at 1-min intervals for 10 min. Once a temperature gradient was established, distribution of fish was again recorded at 1-min intervals for 10 min. Thereafter distribution was recorded at 1-min intervals for 10 min every 30 min until 10 sets of observations were made. The temperature of each zone was recorded before and after each set of observations, and the mean was taken. An effort was made to maintain a gradient of about 1 degree C from one zone to the next. Temperature for a given zone was not held constant over all of the 10-min observation periods. Hence for a given experiment temperature range usually exceeded the number of zones in the experimental tank.

Often after the initial temperature gradient was established, fish congregated near the surface or bottom. When this occurred it was assumed the thermal gradient was not adequate and observations were discarded. Usually this was overcome by the second or third observation period. Once an adequate thermal gradient was established fish generally congregated within a narrow range of temperatures regardless of the vertical location within the tank. There was no evidence that distribution of fish was influenced by vertical gradients of light, pressure, and dissolved gases.

Distribution of fish was recorded before the establishment of a thermal gradient as an indication of bias resulting from such factors as irregularities on the inner surface of the tank. Control distributions varied widely among experiments. Hence it was presumed a better estimate of control distribution would be made by pooling observations from all experiments.

The overall mean numbers of fish recorded in the various zones of the tank for 10 observations, each on 10 fish at 1-min intervals, were

Zone	1(top)	2	3	4	5	6	7	8	9	10	11	12
No. fish												
Mean	5.6	7.3	6.7	7.8	11.4	11.3	12.3	11.4	6.7	7.0	5.0	7.5
SD	6.4	7.1	5.4	5.2	4.4	6.5	6.1	5.2	3.9	4.0	3.9	7.2

Values for the pooled control distribution were subtracted from those for each set of observations on fish in an adequate gradient. Frequency distribution in relation to temperature was calculated by summation of all adjusted observations made at a given temperature from the appropriate sets of observations. Totals were divided by the number of sets of observations to bring results among experiments to a common standard. The mode of the frequency distribution was taken as the temperature preferendum (Pitt et al., 1956). The mean weight of fish and date of experiment for each acclimation temperature and salinity are given in Table 1. The adjusted

temperature distribution frequencies for each acclimation temperature and salinity are available on request.

TABLE 1. Weights of the groups of fish used and date of each experiment in the determination of temperature preferenda of *Tilapia nilotica* acclimated to different temperatures and salinities. When the temperature preferendum was determined more than once for a given temperature and salinity acclimation, the mode of the combined frequency distribution was used.

Acclimation temp (*C*)	Date	Weight (g)		Temp preferendum (adjusted mode) (*C*)
		Mean	SD[a]	
		Salinity 0‰		
15	Oct. 1967	[b]		33
20	"	[b]		33
25	"	[b]		33
30	Dec. 1967	10.4	3.7	30
35	Dec. 1967	9.4	2.8	28
35	Apr. 1967	10.0	3.4	28
35	Dec. & Apr.			28
		Salinity 7.5‰		
15	Dec. 1967	5.8	2.7	33
20	"	5.6	3.6	33
25	"	5.9	2.8	33
30	Jan. 1967	11.7	2.8	28
30	Apr. 1967	9.2	3.2	29
30	Jan. & Apr.			29
35	Dec. 1967	11.4	2.8	28
		Salinity 15.0‰		
15	Dec. 1967	6.0	2.0	30
20	Jan. 1968	6.2	2.4	33
25	Jan. 1968	6.1	2.0	29
30	Feb. 1968	9.5	2.7	29
30	Apr. 1968	11.5	4.0	28
30	Feb. & Apr.			28
35	Mar. 1968	11.1	4.0	27
		Salinity 22.5‰		
15	Feb. 1968	6.2	2.0	29
20	Mar. 1968	6.7	1.9	32
25	Mar. 1968	10.2	2.4	32
30	Feb. 1968	9.7	2.2	29
35	Feb. 1968	9.6	5.1	28
35	Mar. 1968	10.8	3.6	27
35	Feb. & Mar.			27
		Salinity 30.0‰		
15	Feb. 1968	6.1	2.4	31
20	"	6.2	2.5	32
25	"	9.6	4.0	31
30	Mar. 1968	9.8	2.8	30
35	Mar. 1968	11.8	2.4	29

[a]SD = standard deviation.
[b]Weight not determined.

RESULTS

In general, maximum temperature preferenda occurred at acclimation temperatures of 20 and 25 C irrespective of salinity. Further, as acclimation temperature was increased above 25 C, temperature preferenda rapidly decreased. Similarly, preferenda declined at salinities other than 0 and 7.5‰ when acclimation temperature decreased from 20 to 15 C.

The temperature preferendum in fresh water (0‰) was 33 C when fish were acclimated to 15, 20, and 25 C (Fig. 1, 2), 30 C when they were acclimated

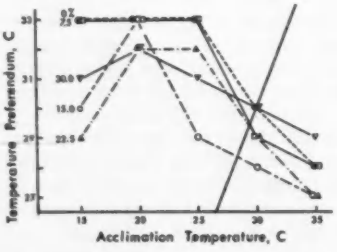

FIG. 1. Modal temperature preferenda of *Tilapia nilotica* after acclimation to different temperatures and salinities. The final preferendum is indicated by the point where a given curve intersects with the 45-degree line through the origin.

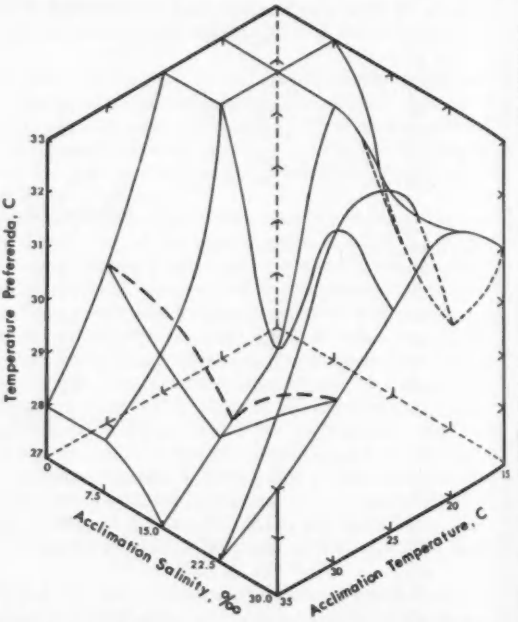

FIG. 2. Modal temperature preferenda of *Tilapia nilotica* in relation to acclimation temperature and salinity. The heavy broken line denotes final temperature preferenda at different salinities.

to 30 C, and 28 C when they were acclimated to 35 C. At 7.5‰, the pattern of the relation between acclimation and the preferendum temperature was similar to that at 0‰. Indeed, at acclimation temperatures of 15, 20, and 25 C, temperature preferenda were the same at the two salinities. For acclimation temperatures of 30 and 35 C, temperature preferenda at 7.5‰ were 29 and 28 C respectively, at each acclimation temperature 1 degree C less than that observed in fresh water.

At 15.0‰, the temperature preferendum increased from 30 to 33 C when the acclimation temperature was raised from 15 to 20 C. With a further increase in acclimation temperature, the preferendum decreased. Among the

salinities examined, the temperature preferendum was generally lowest at 15.0‰ for acclimation temperatures between 25 and 35 C. The one exception occurred at an acclimation temperature of 35 C, where the preferendum at 15.0 and 22.5‰ was 27 C.

At 22.5 and 30.0‰ the patterns of the relation between acclimation and preferendum temperatures were similar. Temperature preferenda increased with acclimation temperature from 15 to 20 C and remained constant at 25 C. As at other salinities, temperature preferenda decreased when acclimation temperature was elevated to 30 and 35 C.

The final preferendum temperature at each salinity was determined from the intersection of the curve of the temperature preferendum with the 45° line drawn through the origin (Fig. 1). Final preferendum temperatures were highest and the same, 30 C, at 0 and 30.0‰. However, between the extremes in salinity, the final preferendum temperature decreased to a low of slightly over 28 C at 15.0‰ with an intermediate value at 7.5 and 22.5‰.

DISCUSSION

Generally, the temperature preferendum for a given acclimation temperature was high at the salinity extremes, 0 and 30‰, and low at intermediate salinities. For example, at 25 C, preferenda at 0 and 30.0‰ were 33 and 31 respectively; at 15.0‰, 29 C. Only at 20 C was the preferendum temperature not less at intermediate salinities than at the extremes. In accord with the general trend was the relation between final temperature preferendum and salinity. Moreover, the influence of salinity on preferenda or final preferenda was similar in pattern to that on oxygen consumption (Farmer and Beamish, 1969). Oxygen consumption of *T. nilotica* for a given sustained swimming speed was high at 0 and 30‰ and lowest at the isosmotic salinity, 11.6‰. Presumably, energy expenditure associated with osmoregulation was least in the absence of an osmotic gradient. Among the salinities followed in the present study, 15‰ was closest to the isosmotic salinity. It is interesting that in the presence of sublethal concentrations of potassium cyanide, a respiratory depressant, Javaid (MS, 1967) demonstrated a decrease in temperature preferenda of several salmonids.

At several acclimation temperatures, the preferendum was not changed by more than 2 degrees C between 0 and 30‰. Often in preferenda studies a shift of 1 or even 2 degrees C is not regarded as significant. However, the similarity in pattern of the relation between preferendum and salinity among most of the acclimation temperatures with that between the final preferendum or oxygen consumption and salinity suggests the few degrees may indeed be of physiological significance.

Of the species for which the relation between acclimation and the preferendum temperature has been described, only sockeye salmon (*Oncorhynchus nerka*) (Brett, 1952) followed a pattern similar to any of the three acclimation type curves found for *T. nilotica*. The curve for sockeye salmon at 0‰ and those for *T. nilotica* at 22.5 and 30.0‰ followed similar patterns.

Final temperature preferenda for *T. nilotica* are in general agreement with optimum temperatures for several activities. Much of the available information on temperature effects in *T. nilotica* has been obtained from pond culture studies. Yashouv and Halevy (1967) suggest that a temperature of 28 C is within the optimum range for growth. Further, Chervinski (1967) indicates reproduction is most satisfactory in the vicinity of 28 C. Laboratory studies on swimming capacity of *T. nilotica* suggest performance is maximum over the range 28–32 C (Fukusho, 1968).

The final temperature preferenda for *T. nilotica*, 28–30 C, are in line with those found for several temperate species particularly within the families Centrarchidae and Cyprinidae. Among the Centrarchidae, the final temperature preferendum was 32 C for largemouth bass (*Micropterus salmoides*), bluegill sunfish (*Lepomis macrochirus*), and pumpkinseed sunfish (*Lepomis gibbosus*) (Hart, 1952). Of the Cyprinidae studied, carp (*Cyprinus carpio*) have a final preferendum of 32 C (Pitt et al., 1956) and goldfish (*Carassius auratus*), 28 C (Fry, 1947).

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Stream Habitat Utilization by Cohabiting Underyearlings of Chinook (*Oncorhynchus tshawytscha*) and Coho (*O. kisutch*) Salmon in the Big Qualicum River, British Columbia

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LISTER, D. B., AND H. S. GENOE. 1970. Stream habitat utilization by cohabiting underyearlings of chinook (*Oncorhynchus tshawytscha*) and coho (*O. kisutch*) salmon in the Big Qualicum River, British Columbia. J. Fish. Res. Bd. Canada 27: 1215–1224.

Habitat distributions of chinook and coho salmon underyearlings in the Big Qualicum River, Vancouver Island, B.C., under controlled flow conditions indicated that at similar sizes their habitat requirements during the first 3 months of stream life were similar. Just after emergence, fry of both species occupied marginal areas in association with bank cover. With increased size, the young fish moved into habitat of progressively higher velocity. However, differences between the species in time of emergence and size evidently resulted in a high degree of spatial segregation. Chinook fry emerged about a month earlier than coho, were larger upon emergence, and grew at a faster rate. Apparently, because of their larger size at a given time, chinook preferred higher velocity locations than coho.

Received July 23, 1969

INTRODUCTION

CHINOOK (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon cohabit many of the larger rivers of south coastal British Columbia. Juveniles of the two species have similar habitat requirements, but may differ in the length of time they spend in fresh water. Coho salmon normally enter salt water a full year after fry emergence (Foerster, 1955). Studies of two coastal fall-run chinook populations in British Columbia (Neave, 1949; Lister and Walker, 1966) have shown that the juveniles migrate seaward within 3 months of emergence.

In this paper, we compare the habitat distributions of underyearling fall-run chinook and coho during the 2–3 months that they cohabit a stream, and we relate it to species differences in size and emergence timing. We conducted the study in 1967 at the Big Qualicum River, Vancouver Island, B.C., the site of a major stream flow control project. The highly stable stream discharge level maintained during the investigation greatly facilitated the study of each species and size-related changes in their habitat requirements.

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STUDY AREA

The Big Qualicum River (Fig. 1) enters the Strait of Georgia 63 km north of Nanaimo. In an attempt to increase the salmon production of this stream, the Department of Fisheries and Forestry of Canada constructed works to

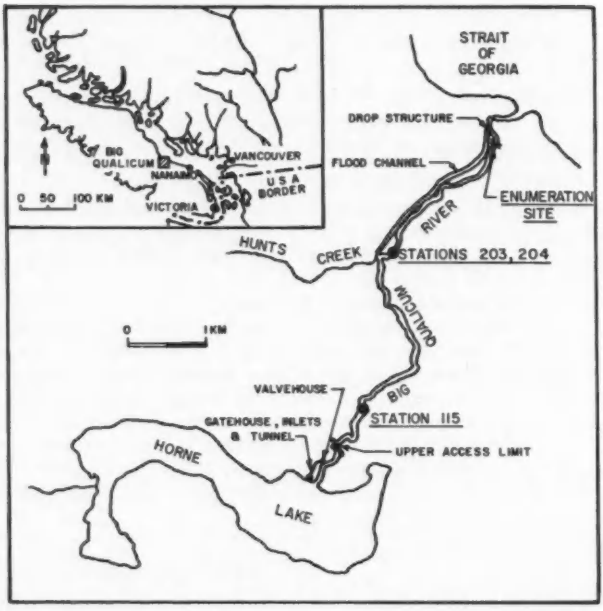

FIG. 1. Lower Big Qualicum River, showing features of the flow control project, seining stations, and counting fence.

permit stabilization of winter flows, increase in the minimum summer flow, and a degree of water temperature control. Lister and Walker (1966) described the physical characteristics of the river and the associated flow control scheme.

Flow control began in the fall of 1963. Control works at Horne Lake (elevation, 120 m; area, 7.8 km^2) provide for regulation of over 90% of the total surface discharge from the watershed. Water is conducted from a gate-controlled intake on the lake 520 m by tunnel to a valvehouse (Fig. 1) where discharge to the lower river is precisely regulated.

During most of the study period, March 2–May 24, river discharge was maintained at about 5.7 m^3/sec; it was then reduced over a 10-day period to 2.8 m^3/sec (Fig. 2). Under these discharge conditions the river was 15–25 m wide at most locations. Mean daily water temperatures increased from 4 C in early March to 15 C in mid-June.

Since 1959, the Department of Fisheries and Forestry of Canada has maintained a count of adult and juvenile salmonid migrants at a fence 700 m upstream of tidal influence (Fig. 1). Most adult chinook and coho enter the river during October. Chinook spawn from mid-October through the 1st week

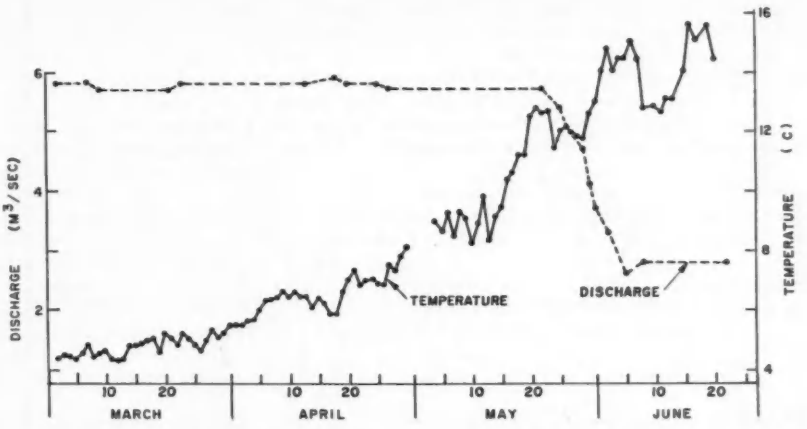

FIG. 2. Daily discharge and mean daily temperature of the Big Qualicum River during the spring of 1967.

of November; peak spawning occurs in the last week of October. The peak of coho spawning occurs during the first half of December, but small numbers of coho spawn from mid-November through January. An impassable falls, approximately 500 m downstream of the valvehouse, restricts spawning to the lower 11.2 km of river. Although their timing differs, most chinook and coho spawn in the upper 4 km of river.

Immediately after emergence in the spring, considerable numbers of chinook and coho fry move downstream past the counting fence (Fig. 3). We do not know whether significant numbers of fry emigrants actually enter salt water, and doubt that coho fry do so. Juvenile chinook pass the counting fence either as fry or as 2- or 3-month-old fingerlings, 70–80 mm long. This study focuses on distribution of underyearlings of the two species mainly in the post-emergence period before seaward migration of stream-resident chinook fingerlings.

METHODS

Under the conditions of virtually constant stream discharge during most of the study, changes in fish distribution can be interpreted as indicating changes in habitat requirements, particularly in terms of water depth and velocity.

To determine seasonal trends in the habitat distribution and size of underyearling chinook and coho we collected fish by daytime seining at weekly intervals from March 7 to May 31, 1967. Permanent reference points were established every 200–300 m along the river and are numbered from 100, at the upper limit of access to salmon, to 290, at the river mouth. Each of the three regular sampling locations used in this study (stations 115, 203, and 204, Fig. 1) included either two or three subareas, which represented gradients in water velocity and depth (Table 1). The subareas were 2–2.5 m wide and 4–15 m long. At station 115 the collection area extended from the stream margin to midstream, approximately 7 m from shore. At stations 203 and 204 the subareas were 2–3 m apart.

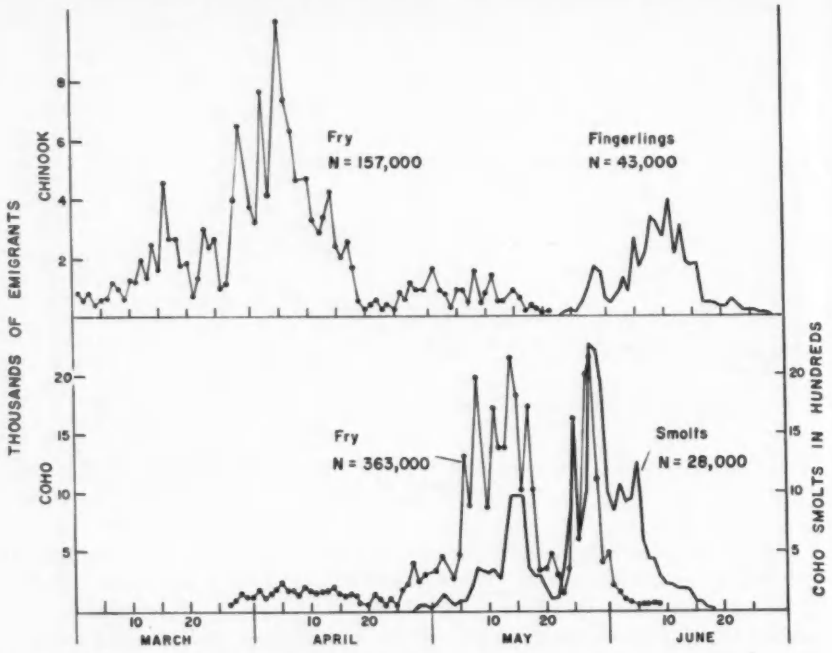

FIG. 3. Timing and abundance of chinook and coho juveniles passing downstream through the fence near the mouth of the Big Qualicum River in 1967.

TABLE 1. Some physical characteristics of seining sites at the discharge (5.7 m³/sec) prevailing during most of the study period, Big Qualicum River, 1967.

Station	Subarea	Area (m^2)	Mean distance offshore (m)	Water velocity (cm/sec)		Depth (cm)		No. observations
				Mean	Range	Mean	Range	
115	Marginal	37	1.7	34	18–49	61	43–85	10
	Intermediate	30	3.7	37	21–49	73	55–98	10
	Midstream	15	6.1	46	21–92	79	61–98	8
203	Marginal	10	1.2	21	10–52	58	40–61	6
	Midstream	7	4.0	34	18–49	58	55–61	6
204	Marginal	8	0.9	24	6–46	64	31–88	8
	Midstream	20	3.7	43	27–58	85	61–95	9

We used two pole seines of nylon netting (8 mm stretched mesh): a two-man seine (4 × 2 m) for general netting, and a smaller seine (1.5 m long) for use under banks and tree roots. Seining was started at a marginal subarea and continued at that location until further effort produced no catch. Total catch in each subarea served as a measure of numbers occupying each type of habitat.

There were two apparent sources of error in the fish collection procedure. High velocity and coarse substrate probably reduced seining efficiency somewhat, and at station 115 we could

not always ensure that fish from an adjacent subarea did not enter the catch. The former source of error would lead to an underestimate of both abundance and average size of larger fish and would apply mainly to the chinook fingerlings. Despite these shortcomings, we consider that seining provided adequate comparative data on trends in distribution and size of the two species.

During the early part of the emergence period, chinook fry were not available to seining during the day. To provide a check on our seining information, on two occasions in March we conducted underwater observations with face mask and snorkel, generally by moving upstream slowly along the stream margin.

Fish size data were based on random samples, usually numbering at least 30 individuals of each species, drawn weekly from the total catch at each of the three regular seining stations and on one occasion at stations 134 and 160. Fork-length measures of live anesthetized (M.S. 222) fish were recorded to the nearest millimeter.

Representative water velocity measures were taken in each subarea with a "Gurley" No. 622 current meter. The average velocity at a given sampling point was calculated from a single observation taken at 4/10 of the vertical distance from stream bottom to surface.

INTERSPECIFIC DIFFERENCES IN SIZE AND EMERGENCE TIMING

Associated with the fact that their parents spawned earlier, chinook fry emerged from the gravel earlier than did coho fry. At the seining stations, peak abundance of chinook fry preceded that of coho fry by almost 4 weeks at stations 115 and 203 and by 6 weeks at station 204 (Table 2). Species differences in emergence timing were also reflected in timing of fry emigration at the counting fence (Fig. 3). The peak of emigration of chinook fry occurred in early April and that of coho fry about mid-May.

Also, chinook fry evidently averaged larger on emergence than did coho fry (43 vs. 38 mm) (Table 3). By the beginning of the period of high numbers of coho fry at the counting fence early in May, chinook had an average length of 47 mm, and thereafter the length distributions of the two species were practically distinct (Fig. 4).

Differences in growth rate evidently increased the size differences between the two species. On the basis of seine samples (Table 3), chinook apparently grew at a higher rate. However, at least three factors complicated the comparisons: continual recruitment of emergent coho fry to the resident population, emigration of larger-than-average chinook in late May, and possible emigration of exceptionally large coho in June. Chinook emigrants captured at the fence were significantly ($P = .01$) larger than residents on May 31 (68 vs. 59 mm) and on June 14 (75 vs. 69 mm). We did not measure emigrant coho fry in 1967, but previous data indicated that after the emergence period exceptionally fast-growing individuals predominated in the coho emigration; however, the coho fry emigration was low during June (Fig. 3).

DISTRIBUTION IN RELATION TO FISH SIZE AND AVAILABLE HABITAT

The smaller fry of both species occupied marginal areas in association with bank cover — particularly in back-eddies, or behind fallen trees, undercut tree roots, and other well-protected areas. Both species preferred locations close to shelter, but adjacent to water of high velocity (40 cm/sec). Subsequently, the largest concentration of fish gradually shifted from marginal to midstream

TABLE 2. Trends in density and distribution of underyearling chinook and coho, as indicated by daytime catches (no./m²) in subareas at three stations during the period of emergence and early rearing.

Date	Marginal		Intermediate		Midstream	
	Coho	Chinook	Coho	Chinook	Coho	Chinook
			Station 115			
Apr. 12	0	0	0	0	0	0
27	0.1	1.1	0	0.7	0.1	4.1
May 5	11.5	3.2	0.8	4.5	0.5	0.9
10	11.8	1.1	3.4	5.8	3.3	5.3
17	12.1	0.1	1.6	0.2	6.3	4.5
24	12.7	0.1	2.5	0.8	11.3	2.6
31	7.6	0.1	10.0	0.5	12.3	2.5
			Station 203			
Mar. 21	0	0.2			0	0
Apr. 12	0.4	1.7			0	0
19	4.2	5.0			0	0
27	10.1	7.2			0	0
May 3	18.3	4.7			0.1	2.3
11	18.2	2.4			0.6	5.1
18	23.1	0.4			1.1	6.9
24	45.7	0.8			5.3	7.0
31	6.0	0			4.3	2.0
			Station 204			
Mar. 28	0.1	2.0			0	0
Apr. 5	0.6	3.4			0	0
12	1.8	1.4			0	0
19	0.1	0.9			0	0.2
27	8.3	1.0			0.4	0.1
May 3	10.8	0.8			0.8	0.2
11	19.8	0.1			3.1	0.1
17	28.8	0.9			4.9	0
24	49.9	0			21.7	1.1
31	21.6	0			7.0	0.5

locations (Fig. 5). Chinook preceded coho in this shift away from the stream margin.

Apparently because of their larger size at any given time, chinook occupied higher velocity locations than did coho and thus were spatially segregated from most underyearling coho. This is shown by data on species composition and fish size in relation to stream velocity, which we obtained during June at four stations representing a considerable range of habitat type (Table 4); at the time of observation, relative abundance of chinook fingerlings was declining because of emigration. In association with increasing velocity and the transition from sand to large boulder substrate, the numbers of coho markedly

TABLE 3. Trends in mean fork lengths of underyearling chinook and coho collected at stations 115 and 203 by daytime seining during the period of emergence and early rearing. Sample sizes are shown in parentheses.

Date	Chinook		Coho	
	Mean length (*mm*)	95% confidence limits	Mean length (*mm*)	95% confidence limits
Apr. 12	42.9(17)[a]	42.0–43.8	–	
19	44.3(37)	43.6–45.0	38.4(32)[a]	37.5–39.3
May 4	47.0(82)	45.3–47.7	38.0(90)	34.6–38.4
18	52.8(86)	51.9–53.7	38.5(89)	38.0–39.0
31	58.6(77)	57.4–59.8	38.8(68)	38.2–39.4
June 14	69.3(39)[b]	67.1–71.5	41.6(79)	40.8–42.4

[a]Considered to represent mean length of early emerging fry.
[b]Sample includes fish from all stations.

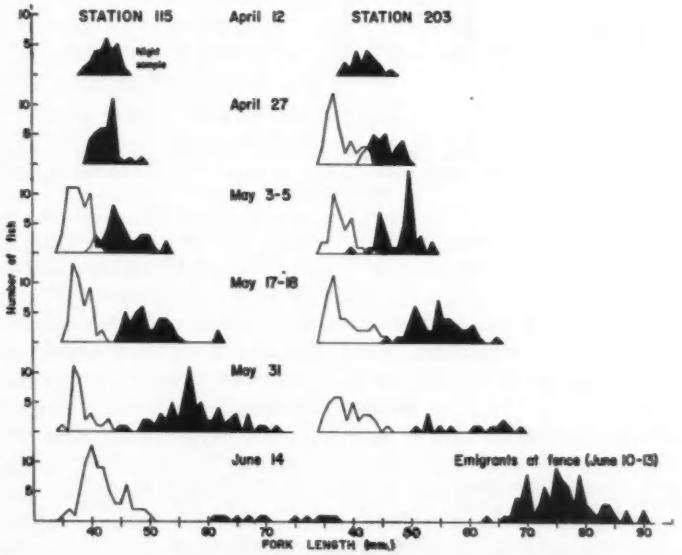

FIG. 4. Length distributions of underyearling chinook (solid) and coho (open) salmon during the period of emergence and early rearing.

decreased, relative and absolute numbers of chinook increased, and the average size of both species tended to increase. Even within a space of 3 m, the species compositions and sizes of fish present differed distinctly, as between subareas at stations 115, 204, and 160.

Although approximately 50% of the migrating chinook fry had reached the counting fence by April 5 (Fig. 3), we could not capture large numbers of

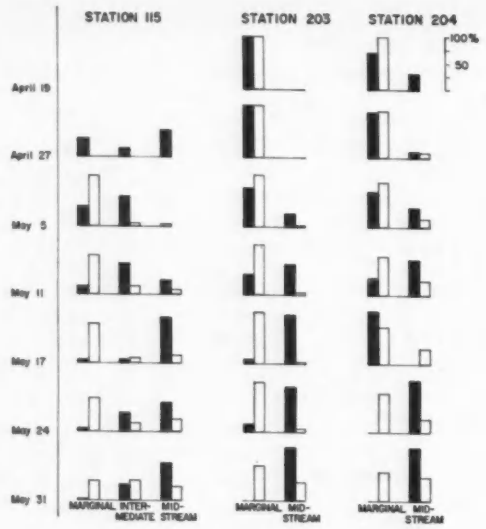

FIG. 5. Trends of distribution (%) of underyearling chinook (solid bars) and coho (open bars) salmon in marginal and midstream subareas during period of emergence and early rearing.

TABLE 4. Numbers and mean lengths of seine-caught underyearling chinook and coho in relation to stream velocity and substrate composition at marginal and midstream subareas of several stations on the Big Qualicum River, June 14–15, 1967.

Station	Water velocity[a] (*cm/sec*) Mean	Range	Substrate composition	No. fish Coho	Chinook	Fork length (*mm*) Coho Mean	Coho Range	Chinook Mean	Chinook Range
			Marginal subareas						
115	12	6–24	Sand and fine gravel (<1-cm diam)	459	1	40.2(33)[b]	35–49	65(1)	
204	21	0–46	Sand and fine gravel	602	0	40.1(36)	35–49		
160	24	0–58	Sand and fine gravel	303	0	43.9(46)	38–52		
			Midstream subareas						
204	40	24–49	Fine and medium gravel	134	3	43.6(28)	40–50	66.3(3)	60–77
160	40	9–58	Medium gravel (1 to 5-cm diam)	13	18	–	–	67.1(17)	59–77
115	46	30–67	2- to 10-cm diam gravel interspersed with 20- to 25-cm diam boulders	46	12	42.5(46)	38–50	72.2(12)	61–82
134	55	30–73	Primarily 15- to 36-cm diam boulders	0	6			72.2(6)	65–81

[a]6–8 observations at each station.
[b]Sample size in parentheses.

chinook in daytime seining until late April (Table 2). However, limited night seining was productive much earlier in the season. Day and night seine catches of chinook fry at station 115 during the period of fry emergence and early rearing were

	Mar. 8	Apr. 12	Apr. 27	May 17
Day (10:30 AM–2:30 PM, PST)	0	0	124	78
Night (8:30–11:00 PM, PST)	5	154	55	47

Evidently, throughout the emergence period the large numbers of chinook fry dispersing downstream remained inactive during the day. Underwater observations confirmed this.

Significant numbers of chinook and coho fry appeared at stations 203 and 204 earlier than at station 115 (Table 2). The former locations afforded considerable cover for the fry under tree roots and stumps whereas at station 115 the shoreline consisted of an open sand bar. Considering the initial hiding tendency of fry, differences among stations in the time of fry appearance are probably related to the extent of marginal cover.

The postemergence sequence of behaviour can thus be summarized as follows: initial hiding (possibly in the gravel); association with bank cover; appearance along open shorelines; and finally, movement into higher velocity locations either along the stream margin or farther out from shore.

DISCUSSION

Although their habitat requirements were similar, chinook and coho were always spatially separated to a large degree. As they grew, both species occupied habitat of progressively higher velocity. Spatial segregation apparently resulted from size differences, which we have related in turn to species differences in emergence timing, size of emergent fry, and growth rate. Our observations indicate that rather small interspecific differences in the timing of life stages could effectively minimize spatial interaction.

Similar segregation of cohabiting species of salmonids has been noted by Everest (MS, 1969). In studies of microhabitat selection by juvenile chinook salmon and steelhead trout (*Salmo gairdneri*), he observed a strong positive correlation between fish size and water velocity at a given feeding station, and postulated that movement of the fish into faster water as they grew was related to availability of insect drift food. He demonstrated that differences in timing of fry emergence also contributed to spatial segregation of the two species and of the different age-groups of steelhead.

Also, segregation of different age-groups of salmonid species in streams has been attributed by other workers to size-related changes in habitat requirements. McCrimmon (1954) and Saunders and Gee (1964) observed that, with increased size, underyearling Atlantic salmon (*Salmo salar*) gradually distributed through riffles with stronger current and coarser bottom material and that by the end of the first growing season the faster-growing underyearlings

might occupy habitat used during the previous summer by yearling parr. Saunders and Smith (1955) noted the spatial segregation of fingerling and older brook trout (*Salvelinus fontinalis*) into riffles and pools, respectively. They also showed that the progressive downstream movement of trout from small headwater tributaries was correlated with increasing size, and that this movement maintained populations of older trout in the larger mainstream areas.

In a review of the literature on factors affecting salmonid distribution in streams, Chapman and Bjornn (1969) state that social interaction is most important as a distribution mechanism among individuals of near-equal size. Size segregation of species, combined with size-related changes in habitat requirements, would therefore tend to reduce the chance of interaction. The results of our study and that reported by Everest (MS, 1969) suggest that species differences in spawning and emergence timing play an important role in segregating ecologically similar species.

ACKNOWLEDGMENTS

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Some Aspects of the Organization of Fish Schools

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VAN OLST, J. C., AND J. R. HUNTER. 1970. Some aspects of the organization of fish schools. J. Fish. Res. Bd. Canada 27: 1225–1238.

The objective of this study was to describe the organization of schools of the pelagic marine fishes *Scomber japonicus*, *Trachurus symmetricus*, *Engraulis mordax*, *Atherinopsis californiensis*, and *Atherinops affinis*. Organization was studied in larval through adult stages by analysis of spatial and angular relations among fish in dorsal photographs of schools.

In all species, schools of young fish, larval and juveniles, were typically less compact and showed greater differences in angular headings than did schools of adult fish. The rate at which school structure changed with size varied among species; it was rapid in *Scomber* and the atherinids and slower in *Engraulis* and *Trachurus*. School organization differed among species at the adult stage. Schools of *Scomber* were typically the most compact and organized; *Trachurus* and *Engraulis* schools were intermediate in their organization and *Atherinops* schools were the least organized.

Spatial and angular measurements were also used to develop inferences regarding the "following reaction" in four of the species. The similarity in angular headings between fish was used as the criterion of following. These analyses showed that the similarities in headings between two fish in a school decreased with the distance they were apart. The headings most alike between adjacent fish in a school were between those in file. These results suggested that the neighbor directly ahead of a given fish in a school is used more frequently as an angular reference for the following reaction than ones to the side.

Analysis of the distances between neighbors in the four species indicated that fish were closer to adjacent neighbors in the file than they were to ones in rank. This suggested that spacing in the horizontal plane might be related to tail movement.

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INTRODUCTION

THE PRINCIPAL CHARACTERISTICS of the organization of fish schools are that the individuals stay together, tend to head in the same direction, maintain even spacing, and the activities of individuals tend to be synchronized. The mechanism for the cohesion and synchronization of a school is considered to be the "following reaction," a concept used by Crook (1961) and others to describe bird flock organization and later applied to fish schools by Hemmings (1966). Cohesion and synchrony of movement develop gradually during early growth (Shaw, 1960) and vary among schooling species at maturity. The purpose of this study was to compare and describe these aspects of school organization for certain pelagic marine fishes at different stages of development by analysis of spatial and angular relations.

Angular differences were used to estimate the extent of the following reaction because when a fish failed to follow it was headed in a direction different

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from the rest of the fish. Measurement of angular differences also provided an estimate of the cohesion and synchrony of movement of the groups because these characteristics are dependent on the following reaction. Measurements of interanimal distances were made to determine if the distances between fish in the schools were species-specific and to determine the extent interanimal distances changed during development. Additional spatial and angular measurements were made to evaluate several hypotheses regarding the mechanism of following.

The species studied were *Scomber japonicus* (Pacific mackerel), *Trachurus symmetricus* (jack mackerel), *Engraulis mordax* (northern anchovy), and two atherinids, *Atherinopsis californiensis* (jacksmelt), and *Atherinops affinis* (topsmelt). *Atherinops* was used in all work with postlarval and larger size classes of atherinids but larval *Atherinops* could not be separated from larval *Atherinopsis* without a postmortem vertebral count and consequently groups of larvae contained both species.

All of these species are schooling pelagic marine fish and all would be considered obligatory schoolers (Breder, 1967). Nevertheless, field observations suggested that even among these comparatively strong schooling fish, differences in school organization existed.

METHODS

Larval fish (21–40 mm) were captured at sea under a night-light, held in large seawater troughs, and fed *Artemia* nauplii at the marine aquarium of the Bureau of Commercial Fisheries Fishery-Oceanography Center, La Jolla, California. Juveniles were captured at sea, held in 4-m diam plastic pools, and fed live or frozen adult *Artemia*. Adult fish were purchased at a bait receiver, held in an 8-m diam pool, and fed frozen adult *Artemia* or chopped anchovy.

Measurements of the spatial and angular relations among fish in schools were taken from two-dimensional dorsal photographs of groups of six fish. To obtain proper photographic resolution and to avoid constriction of the schools, container size varied with the size of the fish photographed. Larvae were photographed in 48 × 60-cm oval or rectangular tanks and adult fish in a plastic pool, 8 m in diameter. Sea water temperature varied seasonally (20 ± 5 C). The 8-m pool was outdoors; the rest of the experimental tanks were placed in soundproof white rooms and were surrounded by tungsten lamps and given a 12-hr light cycle.

Groups were acclimatized in test containers for 2 days and filming of each group began on the 3rd day. Photographs were taken at 1-min intervals from 9:00 AM to 4:00 PM and the fish were fed at 12:00 AM–1:00 PM. We analyzed 50 or more of the photographs taken of each group. In each photograph the X–Y coordinates for head and tail of each of the six fish were determined and the mean distance to nearest neighbor and mean angular deviation of the school were calculated from the coordinate data.

The *mean distance to the nearest neighbor* is the average of the distances from each fish in a school to its nearest neighbor. The measurement is made between the two closest points on the midline of the bodies of the two fish, regardless of their orientation. The same measurement is used twice if two individuals are closer to each other than to any other individual in the school.

The *mean angular deviation* is a measure of the differences in orientation among fish within a school. To calculate the index, the heading of each fish, in degrees, is determined. Then the resultant direction of the school is computed by assigning each fish a value of one and adding the headings vectorially. The number of degrees each fish deviates from the resultant direction of the school is determined and a mean calculated. This value is the mean angular deviation.

Additional indices to be described in a later section were used to evaluate several hypotheses regarding the following reaction in fish schools.

In juvenile *Trachurus* schooling behavior has been reported to change during periods of hunger and food searching (Hunter, 1966). To account for and measure this possible source of variability in all groups, we analyzed 25 photographs taken 2 hr after feeding and 25 taken after a 24-hr deprivation period. In three out of four groups of juvenile *Trachurus* (< 70 mm standard length (SL)) the interanimal distances decreased after feeding (lower distance to nearest neighbor values — Mann-Whitney U test — $P = .05$) in accordance with Hunter's data. However, larger *Trachurus* and all size groups of *Scomber*, *Engraulis*, and the atherinids showed no consistent pattern. For adult *Scomber* additional data were taken. Two groups of adult *Scomber* (350 mm SL) were tested over deprivation periods of 2–9 days, but no relation between food deprivation and distance to nearest neighbor or mean angular deviation was found. Schools of *Scomber* after 9 days of food deprivation were the same as ones recently fed. Thus the effects of food deprivation on school structure were limited to small juvenile size classes of *Trachurus*. Consequently, in the following sections we have combined the data from before and after feeding to increase our sample size.

CHANGES IN SCHOOLING WITH SIZE

The objective of this analysis was to compare school structure among different size classes of fish varying from larvae to adults.

The data were segregated into size classes of 20 mm SL on the basis of the mean length of the fish in each group. The numbers of groups of six fish and the numbers of photographs analyzed for each of the size classes are shown in Table 1. Mean angular deviation and mean distance to nearest neighbor were

TABLE 1. Number of groups tested and photographs analyzed for each length class.

Genus	Length class (*mm*)	No. groups	No. photographs analyzed
Scomber	41–60[a]	2	100
	61–80	1	50
	81–100	1	50
	101–120	1	50
	350	2	450
Atherinops & *Atherinopsis*	21–40[a]	2	100
Atherinops	41–60	2	100
	61–80	1	50
	121–140	1	50
Trachurus	41–60	2	100
	61–80	2	100
	81–100	4	200
	101–120	2	100
	121–140	1	50
	250	2	150
Engraulis	21–40[a]	2	100
	61–80	4	300
	81–100	1	100
	101–120	2	150

[a]Larval fish.

calculated for each photograph analyzed. To facilitate comparisons between fish of different lengths the mean distance to nearest neighbor was divided by the mean body length of the group and is presented as the number of body lengths between nearest neighbors (Breder, 1954).

In all species the mean angular deviation of the school and the mean number of body lengths between nearest neighbors were less in schools of large fish than in schools of small ones (Fig. 1). Thus, schools of large juveniles and

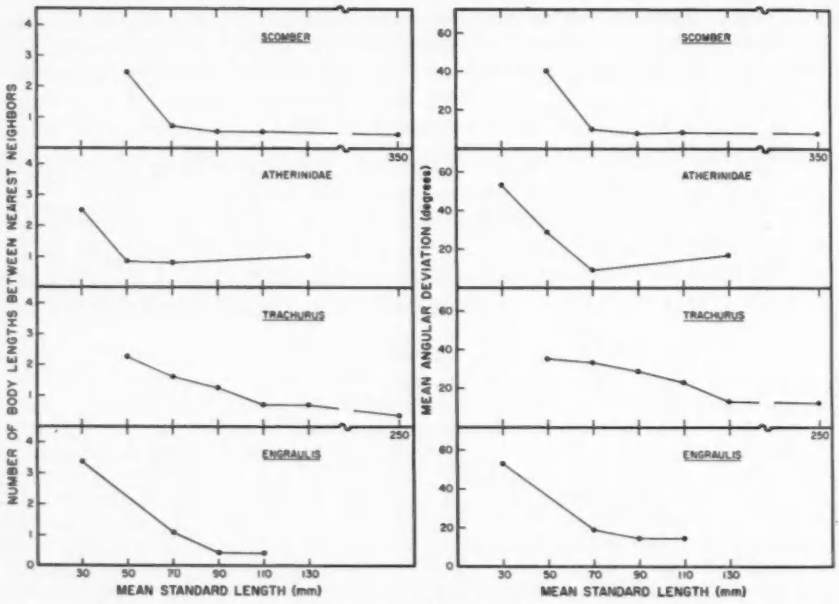

FIG. 1. Relation between school structure and length in *Scomber*, Atherinidae, *Trachurus*, and *Engraulis*. Left, distance between nearest neighbor in body lengths, and right, mean angular deviation of the school. Midpoints of each length class are shown on abscissa, each point is the median of the values from all photographs analyzed for the particular length class. The number of photographs analyzed per length class are shown in Table 1.

adult fish were more compact and less variable in the directional heading among individuals than were schools of larval and small juvenile fish. The rate at which schooling changed with size differed among species. It was more rapid in *Scomber* and in the atherinids than in *Trachurus* and *Engraulis*. In *Scomber* for example, the schooling indices from groups in the 70-mm size class were the same as those for adults, whereas in *Trachurus* schools in the 70-mm size class were less compact, and the individuals were less uniformly headed than were adults.

Comparisons of the frequency distributions of mean angular deviation and mean distance to nearest neighbor to random distributions showed in all species that the schooling indices were nonrandom (chi-square median test,

$P = 0.01$). Thus, the earliest stages of the development of schooling (see Shaw, 1960) had already occurred in the youngest specimens photographed. These data, therefore, do not document the entire development of schooling but only the later portion of it. They clearly show, however, that the development of the compact and integrated school of adult pelagic fish is a gradual process that may extend through a considerable portion of juvenile life.

That well-integrated schools occur late in the development of some of the species, for example *Trachurus*, does not imply that their younger stages could not also form well-integrated schools. Under certain circumstances such as disturbances near or in the tank, fish of all sizes momentarily formed well-integrated schools. For example, we filmed one group of *Trachurus*, 64 mm SL, when a predator was in the same tank. The median mean angular deviation for 25 photographs was 10° and the median distance to nearest neighbor was 0.25 SL. Thus, the presence and movements of a predator temporarily produced in a school of 64 mm *Trachurus* structural characteristics normally associated with the schools of adults. In the experiments presented in this section photographs were taken over relatively long periods and minor disturbances that led to the brief formation of well-organized schools probably had little or no effect on the medians. Thus, our measurements of spatial and angular relations provided an estimate of the typical level of school organization for a given size class under monotonous laboratory conditions. That schools of young fish were less organized than schools of adult fish implies that young fish did not typically follow the movements of their schooling companions as consistently as did older ones.

Differences in experience or maturation of organ systems might explain the differences in the following tendency and in interfish distances among juvenile and adult fish. We are more inclined to believe, however, that these differences were an adaptation to the higher food requirements of juvenile fish. Owing to the energy required for growth and to lower energy reserves, young fish must feed and search for food more often than adults. The nearly continuous maintenance of uniformly headed, compact schools as occur in the adult stage probably would not be adaptive in younger fish because it would probably interfere with feeding and food search. In sum, obligatory schooling fish (Breder, 1967) appear to pass through a facultative schooling stage, the adaptive significance of which may be to allow more flexibility and time for feeding and food search than is possible in the more rigidly controlled obligatory condition.

SPECIFIC DIFFERENCES IN SCHOOL STRUCTURE

The distances separating adult individuals were not species-specific but some differences existed. The medians of the mean distance to nearest neighbor in adult *Scomber*, *Trachurus*, and *Engraulis* were the same, 0.4 SL, whereas the median for adult *Atherinops* was 1.0 SL and was different from the other three species (chi-square test, $P = 0.01$). Interfish distances in fish schools have been measured by a number of other authors (Table 2) but their techniques and numbers of observations varied so widely that it is not possible to separate

TABLE 2. Interfish distances for schools of various genera of fishes.

Genus	Fish length (*mm*)	Distance between nearest neighbors (*body lengths*)	Author and date
Menidia	7–10	18.0–72.0	Shaw (1960)[a]
Tilapia	11	1.3	Dambach (1963)[b]
Menidia	11–12	0.7–2.5	Shaw (1960)[a]
Menidia	12–16	0.4–2.0	Shaw (1960)[a]
Ameiurus	18	0.20	Breder (1954)[c]
Mugil	25	0.17	Breder (1965)[c]
Atherina	51	0.18	Breder (1954)[c]
Jenkinsia	58	0.25	Breder (1954)[c]
Harengula	75	0.6	Cullen et al. (1965)[b]
Trachurus	68	.25	Frightened by predator; current study
Engraulis	101–120	0.4	Current study
Atherinops	121–140	1.0	Current study
Sardinella	150	0.16	Breder (1954)[c]
Trachurus	250	0.4	Current study
Scomber	350	0.4	Current study
Sarda	570	>1.0	Magnuson & Prescott (1966)[a]
Thunnus	1800	0.5	Breder (1967)[c]

[a]Estimated value.
[b]Three dimensional analysis.
[c]"Minimum normal swimming distance between side-to-side fishes" Breder (1954).

specific differences from differences in technique and accuracy of measurement. The values given by Breder (1954, 1967) are lower than other values probably because they are, according to the author, the "minimum normal swimming distance between side-to-side fishes." The most precise measurement of interfish distance was made on schools of *Harengula* by Cullen et al. (1965) who used a three-dimensional analysis. Their estimate of mean distance to nearest neighbor of 4.4 cm (measured between heads), estimated by us to be about 0.6 body length, is relatively close to our value of 0.4 SL determined for adult *Scomber*, *Trachurus*, and *Engraulis*, especially when one considers that our measurements are biased downward because of the omission of the vertical plane and because our measurements were made between the nearest points on the midlines of the body whereas theirs were made between heads. Considering all sources of variability the measurements for mature pelagic marine fish are surprisingly close. We suspect that the mean distance to nearest neighbor for most pelagic schooling fish under monotonous conditions is generally about half a body length.

In the current study the median mean angular deviation for adult *Scomber* was 8°, *Trachurus* 12°, *Engraulis* 15°, and *Atherinops* 17°. The frequency distri-

butions of mean angular deviation for *Trachurus* and *Engraulis* were not statistically different from each other but both were different from *Scomber* and from *Atherinops* (chi-square test, $P = 0.01$).

A ranking of the four groups by their mean angular deviation would agree with our subjective impression of the relative extent of school integration in the four groups. All the species used in this study formed well-integrated schools but their schools differed in general appearance. They also differ in the kinds of habitats they most frequently occupy in the ocean. Atherinids most commonly occur near the shore in shallow water, *Trachurus* and *Engraulis* occur in these areas but also commonly occur offshore, and *Scomber* occurs offshore. Ranking of these species or related ones by visual pigment absorption maxima also indicates that *Scomber* and *Trachurus* are adapted to blue offshore water whereas the atherinids and possibly *Engraulis* are adapted to green inshore water: *Scomber* 491 mμ; *Anchoa compressa* 507–508 mμ (a species in the same family as *Engraulis*); *Trachurus* 495 mμ; and *A. affinis* and *A. californiensis* 508 mμ (Munz, 1957). These relations suggest that the tendency not to stray from the school appears to be more strongly developed in fish adapted to the offshore environment. This conclusion is consistent with similar conclusions drawn by Breder (1967) and others, that, in general, schooling is more strongly developed in species that inhabit offshore environments.

Few measurements of the angular deviation of fish in schools exist: Clark (1963) estimated from aerial photographs that the greatest angle of deviation of individual rays (probably *Rhinoptera bonasus*) from the general direction of the school was 42°; Cullen et al. (1965) found that 44% of their measurements of mean angular deviation of schools of *Harengula* were 5° or less; Hunter (1966, 1968) measured the mean angular deviation of juvenile *Trachurus* and obtained values similar to the ones presented here; and Shaw (1969) considered an angular difference of 26° or less to be typical for schools of "parallel-oriented" *Caranx*. The lack of angular measurements is unfortunate because our results suggest that measurement of angular differences is a more useful criterion of specific differences in school organization than are inter-animal distances.

ASPECTS OF THE FOLLOWING REACTION

The object of this analysis was to develop inferences regarding the mechanism of the following reaction in fish schools from spatial and angular relations within the school. The following reaction in moving fish schools involves an angular component and a velocity component. A fish would be unable to school either if it failed to increase velocity when other fish did or if it failed to execute a course change when others did. Because this analysis was of still photographs of schools only the angular component could be examined. The velocity component has been examined using cinephotography in another study (Hunter, 1969).

In this analysis 450 photographs of *Scomber* (350 mm mean SL), 200 photographs of *Atherinops* (80 mm mean SL), 300 photographs of *Engraulis*

(68 mm mean SL), and 700 photographs of *Trachurus* (117 mm mean SL) were used. The data for the first three species were drawn from those used in the preceding section (see Table 1) but the data for *Trachurus* was taken from a previous study (Hunter, 1968) and reanalyzed with the current objectives. The conditions under which *Trachurus* schools were photographed in the former study were the same as in the present one except photographs were taken at dawn under dim illumination as well as during the day but only the daytime photographs were used in the current analysis.

Two new indices were used in the analysis, separation angle and direction to nearest neighbor. *Separation angle* was considered to be a measure of the angular component of the following reaction. It was assumed that the extent of similarity in heading between two fish in the school was related, to the extent that one used the other as an angular reference for the following reaction. *Direction to nearest neighbor* is the angular position of the nearest neighbor relative to the position of the considered fish. The angular measurement was made between the tips of the snouts of the two fish, the heading of the considered fish was made equal to a compass heading of 0, and the position of the neighbor was calculated relative to this heading. Values greater than 180° were subtracted from 360°. If the neighbor was directly in front of the considered fish the direction to nearest neighbor was 0°, if directly behind it was 180°, and if their snouts were perfectly aligned side by side it was 90°.

The first question asked was whether or not a given fish tended to adjust its heading in relation to the nearest fish or adjusted its heading in relation to that of the group as a whole. For each fish in the group of six the separation angle between the fish and its nearest neighbor was compared with the average heading of the remaining five fish taken as a group. In all species except *Trachurus* the separation angle between the considered fish and its nearest neighbor was lower than that between the considered fish and the average of the headings of the other fish in the school (Table 3). In other words a greater similarity

TABLE 3. Average separation angle between nearest neighbors and the average separation angle between an individual fish and the mean direction of the rest of the school taken as a unit.

Genus	Separation angle between nearest neighbors (*degrees*)	Group separation angle (*degrees*)
Scomber	14	16
Atherinops	31	33
Trachurus	26	22
Engraulis	29	36

existed between the heading of a given fish and its nearest neighbors than between a fish and the average heading of the group. These data suggested that a fish nearby had more effect on the angular adjustment of a given fish

than did more distant ones, and that a fish did not respond to all members of the group equally.

To discover the extent to which distance between fish influenced separation angle, we segregated the distance between fish into classes of 0.1 body length and calculated the mean separation angle for each distance class. In *Trachurus* the deviation in heading between two fish was correlated with the distance between them, that is the closer together the fish were in the school the more similar were their headings (Fig. 2). Scatter plots for *Engraulis* and *Scomber*

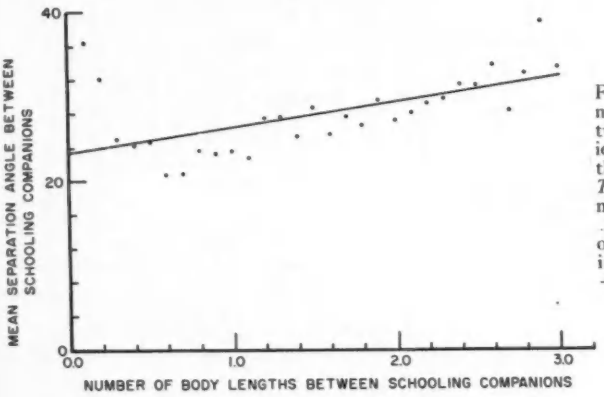

FIG. 2. Relation between mean separation angle between a schooling companion and the distance between them in body lengths for *Trachurus*. Points are the mean separation angle for .1 body length increment of distance between schooling companions (Y = 2.7X + 23.8, R = .57), N = 21,000.

were similar to the one for *Trachurus* and all yielded significant Spearman r_s correlation coefficients (*Trachurus* 0.57, *Engraulis* 0.47, and *Scomber* 0.81), but the data for *Atherinops* was insufficient to make a meaningful comparison. An interesting trend in the scatter plot for *Trachurus* and one that also occurred in the plot for *Scomber* was that at interanimal distances of 0.1–0.2 length the mean separation angle appeared to increase. This could be interpreted to mean that fish tended to avoid interanimal distances of less than 0.2 body length.

The objective of the next step in the analysis was to determine whether or not an individual fish showed any directional preference in the fish used. Differences in separation angle were used as the criterion for directional preference. In this analysis we measured the direction to nearest neighbor, the separation angle between nearest neighbors, and the distance in body length between nearest neighbors.

In all species the mean distance and mean separation angle to nearest neighbors were less when the neighbor was in front of or behind the considered fish than when the neighbor was to the side of the considered fish (Fig. 3). For statistical comparison we grouped the raw data into three 60°-categories of direction to nearest neighbor. Chi-square comparisons of distance and separation angle to nearest neighbor among the three categories of direction to nearest neighbor showed that the front (0–60°) and rear positions (121–180°)

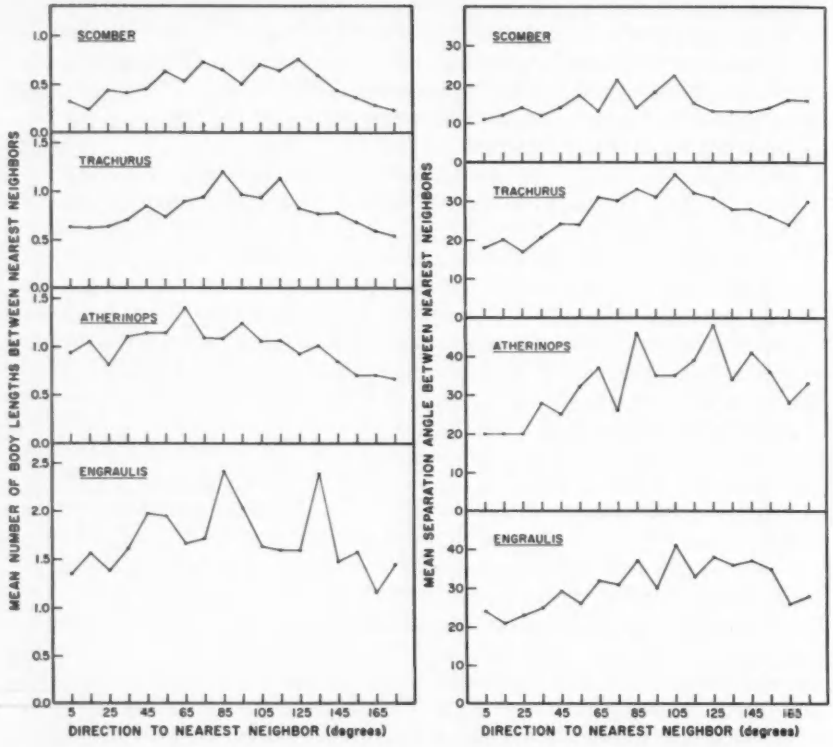

FIG. 3. Mean number of body lengths between nearest neighbors (left) and mean separation angle between nearest neighbors (right) as a function of the direction to the nearest neighbor. Lines connect the means for each 10°-interval of direction to nearest neighbor. The range of N per 10° interval was 65–248 for *Scomber*, 134–376 for *Trachurus*, 39–97 for *Atherinops*, and 40–178 for *Engraulis*.

were different in distance and separation angle from the side positions (61–120°) ($P = 0.01$). Thus, fish approached more closely the neighbor that was ahead of them and maintained a heading more similar to that fish. These data suggest, therefore, that the neighbor ahead of a given fish in a school tended to be used more frequently as an angular reference for the following reaction than did neighbors to the side, and that fish tended to swim closer to the neighbor ahead of them.

As a final step in the analysis we wished to determine whether or not any particular angular position of fish in the schools of six fish occurred with any greater frequency than other positions. For this analysis we determined for each fish in a photograph the direction to every schooling companion. These data were summarized by calculating the frequency in percent that neighbors occurred in each of 18 angular classes of direction to schooling companions

(Fig. 4). If a school were circular in the horizontal plane the numbers of neighbors occurring in each angular class would be expected to be equal. Schools of *Scomber*, *Trachurus*, and *Atherinops* did not differ from a circular shape

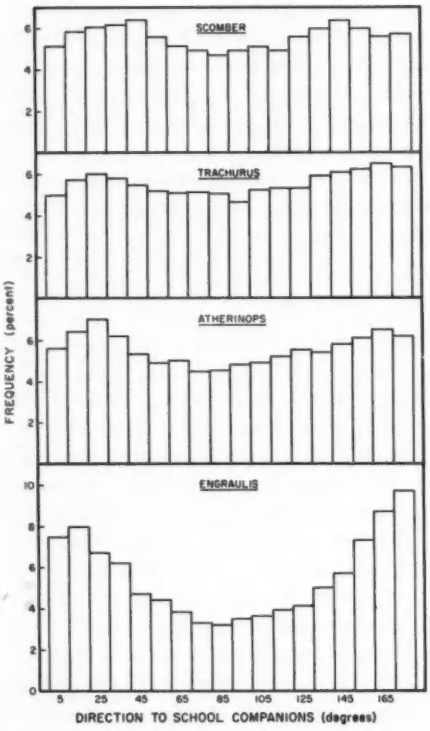

FIG. 4. Frequency of occurrence of all schooling companions (in percent) at various angular positions. The range of N per 10°-category of direction to schooling companions was 627–859 for *Scomber*, 994–1367 for *Trachurus*, 268–391 for *Atherinops*, and 291–872 for *Engraulis*.

although there existed a slight tendency for more neighbors to occur at diagonal positions. *Engraulis* schools, on the other hand, were not circular (chi-square test, $P = 0.05$). In the *Engraulis* schools more neighbors occurred in the front and the rear of the school so that they tended to be slightly elongated in the direction of movement.

DISCUSSION

Our approach in the last section was to search for broad trends in the data. Most important of these was that the heading of a fish in a school was more similar to that of fish swimming ahead or behind it than to that of fish swimming to the side. The implication is that fish ahead of a given fish are more frequently used as an angular reference for the following reaction than are ones to the side. It would be unrealistic to propose, however, that the fish in front of a given fish was the only one used as an angular reference. This

would mean that angular changes by only the fish in the front rank of a school could affect the actions of the rest of the school, which is certainly not true. Other experiments (Hunter, 1969) have shown that *Trachurus* will respond to a speed increase by any fish in visual range. Similarly we believe that a fish can respond to a course change by any fish in visual range, but they tend to rely more frequently on the headings of the fish directly ahead. This interpretation seems reasonable because small changes in direction in the horizontal plane are more readily detected when a fish is viewed from behind than when they are seen laterally and also because the fish in front would often be within the binocular field of the viewer.

Another trend in these data was that fish were closer to the neighbor in front than to the one to the side. This trend suggests that interanimal distances in the horizontal plane may be related to tail movement. In a moving fish school more space would be needed to the side than in front of a fish because space to the side must be sufficient for movement of the tail in the horizontal plane whereas a fish could approach the fish ahead more closely without interfering with the swimming of that fish. This interpretation rests on the assumption that our measurements of spatial arrangements in the horizontal plane were not distorted too greatly by differences in vertical spacing. We limited this source of error by use of shallow containers and small groups of fish. The fish in the school were usually spaced out in the horizontal plane and were not beneath one another but the extent of the difference in vertical position among fish was unknown. Nevertheless, we feel it is of value to consider some of the relations that might exist between tail movement and interfish distance.

The minimum space in the horizontal plane that could exist between the sides of schooling fish would be a function of tail beat amplitude and the relative angular position of the fish. The minimum possible lateral space between neighbors to the side would be lower if the fish consistently occupied diagonal positions than if they were perfectly aligned because the areas of tail movement would not overlap. In the fish we studied, however, the tendency to occupy diagonal positions in the horizontal plane was slight if it existed at all. The distributions of angular positions except in *Engraulis* did not differ from a circular distribution. This was not surprising because typically school structure is dynamic, all positions are in flux, and fish freely change position. To allow for frequent position changes in the direction of movement lateral spacing in the horizontal plane must be sufficient for free movement of the tail regardless of the angular position of the fish. Thus the amplitude of the tail beat may be the most important variable in determining the minimum lateral space.

Tail beat amplitude is the maximum extension of the tail on both sides of the body axis measured perpendicular to the axis to progression and is usually expressed as a proportion of the total length of the fish. During steady swimming at nearly all velocities tail beat amplitude is a constant proportion of body length (L) and appears to vary little among species. The mean amplitude for dace is 0.183 L, for trout 0.174 L, for goldfish 0.202 L (Bainbridge, 1958), for *Scomber* 0.221 L, for *Trachurus* 0.235 L, and for *Engraulis* 0.185 L (J. R.

Hunter, unpublished data). Thus, if minimum lateral spacing in schools were a function of amplitude, one would expect it to be nearly the same in all fish schools, that is the minimum lateral space between body axes would be about 0.2 L.

The average lateral space in the school of *Trachurus* frightened by a predator was about 0.23 total length, which seems close to the tail beat amplitude since an average value would be expected to be higher than the minimum. The values presented for average minimum distance between parallel swimming fish given by Breder (1954) ranged from 0.16–0.25 L. If one assumes the maximum width of the fish studied to be 0.1 L, the range of lateral space between body axes would be 0.26–0.35, which again seems close to the tail beat amplitude during steady swimming.

Other than the one measurement on the frightened school of *Trachurus* the spacing measurements presented in this study were considerably above the minimum of 0.2 total length. The average lateral space in fish schools probably approaches this minimum value only when the school is stimulated by a predator or other fright-inducing stimulus. Under typical conditions the mean distance to the nearest neighbor, an average of interfish distance at all angular positions, was 0.4 SL for adult *Scomber*, *Engraulis*, and *Trachurus*. In adult *Scomber* the number of observations was sufficient to make separate estimates at different angular positions. When *Scomber* were nearly parallel (separation angle = 85° in Fig. 3), the mean space between fish adjusted from standard to total length (TL) was 0.55 TL or roughly twice the tail beat amplitude during steady swimming. Tail beat amplitude does not remain constant during acceleration, however, but increases (Gray, 1968). When *Trachurus* or *Scomber* accelerate they execute a few very high amplitude beats and then gradually decrease amplitude to the level used for steady swimming. In *Scomber* these initial beats are 0.4–0.5 TL (J. R. Hunter, unpublished data). Thus, lateral spacing in adult *Scomber* and possibly also in adult *Trachurus* and *Engraulis* may be adapted to accommodate the high amplitude tail movements required for sudden acceleration. It follows from this line of reasoning that if lateral space in the horizontal plane were less than that needed for acceleration, the school might not be able to accelerate without a loss of integration.

The relations we have suggested that may exist between tail movement and spacing are speculation but sufficient evidence exists perhaps to warrant their examination. Of particular interest would be the study of changes in spacing, and the rate of change of angular positions during steady swimming, during acceleration, and while the school is responding to a predator. The study would be greatly improved if a simple and rapid method could be developed for measurement of spatial relations simultaneously in three planes. Three-dimensional measurements of schools have been made (Cullen et al., 1965) but the methods appear to be too cumbersome for an extensive study on spacing in fish schools.

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Long-Term Changes in the Limnology and Macrozooplankton of a Large British Columbia Lake[1]

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ZYBLUT, E. R. 1970. Long-term changes in the limnology and macrozooplankton of a large British Columbia lake. J. Fish. Res. Bd. Canada 27: 1239–1250.

Macrozooplankton distribution and abundance as well as limnological conditions were compared during 1949 and 1964 in Kootenay Lake, a large oligotrophic lake in southeastern British Columbia. The standing crop of macrozooplankton increased two- to threefold over 15 years. Changes in physical–chemical limnological conditions, such as a decrease in depth of light penetration, and increases in *p*H, specific conductivity, dissolved nutrients, and phosphates also occurred, indicating an increase in productivity.

The most noticeable change in macrozooplankton was a shift in cladoceran species composition in the north arm of the lake, formerly dominated by *Daphnia* but now by *Diaphanosoma*. Size-selective predation, differences in food availability, and competition for food with *Diaphanosoma* and *Mysis relicta* are suggested to be responsible for changes in the spatial distribution of *Daphnia* since 1949.

Examination of changes in midsummer zooplankton abundance between successive years (1964–66) indicated that total zooplankton was least abundant in 1964. Differences in abundance during this period were small when compared with those between 1949 and 1964.

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LIMNOLOGISTS have long been aware of eutrophication, a natural and gradual aging process that changes lakes from deep, cold-water forms, low in nutrients and primary productivity, to shallower, warmwater lakes with high nutrient content and primary productivity. Recently they have found that natural eutrophication can be greatly accelerated by man. This is true not only for small lakes but large lakes such as Lake Erie and Lake Michigan (Beeton, 1961, 1965; Davis, 1964; Anonymous, 1967; Verduin, 1964), the Untersee of Lake Zurich (Hasler, 1947), and Lake Washington near Seattle (Edmondson et al., 1956; Edmondson, 1959). Accelerated eutrophication is usually a result of nutrient enrichment from domestic sewage, industrial developments, and drainage from nutrient-rich agricultural areas.

During recent years large "blooms" of phytoplankton have been observed in Kootenay Lake that were not known to occur 15 years ago. An accelerated process of eutrophication may be occurring, possibly as a result of industrial

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developments (mining and fertilizer plant operations) and from drainage of nutrient-rich agricultural areas.

This paper documents changes in the distribution and abundance of macrozooplankton since 1949 and relates these to changes in limnological conditions associated with eutrophication. Short-term changes in macrozooplankton abundance are examined between 1964, 1965, and 1966.

DESCRIPTION OF THE LAKE

Kootenay Lake lies in southeastern British Columbia (Fig. 1) approximately 27 km from the Canada–United States border. The lake runs on a north–south axis for 105 km with an arm projecting westward for 34 km. The

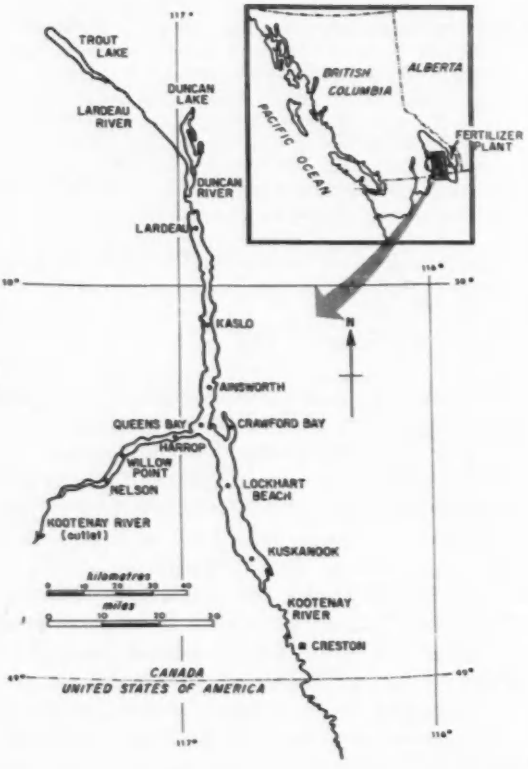

FIG. 1. Location of Kootenay Lake and sampling stations.

main lake has a maximum width of 6.4 km and a maximum depth of 155 m with a mean depth of 109 m. The west arm is shallower, having a maximum depth of 45 m and a mean depth of 13 m.

Two large rivers flow into Kootenay Lake. The principal river, the Kootenay, entering the south end of the lake (Fig. 1), drains an extensive agricultural

area (Brink and Farstad, 1949) and contributes large amounts of silt to the south arm during spring runoff (Northcote and Larkin, 1963). A large fertilizer production plant is situated on St. Mary's River, a tributary of the Kootenay River (Fig. 1, inset). The Duncan River, entering the north arm of Kootenay Lake is generally less turbid and much lower in dissolved nutrients than the Kootenay River.

METHODS

Temperatures were measured with a bathythermograph to a maximum depth of 61 m in 1949 and to 135 m in 1964. Transparency was measured with a standard 20-cm diam Secchi disc.

Location of sample sites are shown in Fig. 1. Zooplankton samples were taken monthly; these were collected with two types of samplers — Winsconsin nets and Clarke–Bumpus samplers. A standard (22.9 cm diam) Wisconsin closing net was used with a No. 10 (0.13-mm) bolting silk net and a No. 20 (0.07-mm) bolting silk bucket similar to that in 1949. Wisconsin total vertical hauls (from near bottom to surface) and hauls from 10 m to the surface were made. Since zooplankton was sparse below 50 m (Zyblut, MS, 1967), calculations were conducted on the basis that all animals were from the upper 50 m.

A Clarke–Bumpus sampler (Clarke and Bumpus, 1950) was fitted with No. 10 nylon netting and used during July of 1964, 1965, and 1966. Tows were taken at 2.5 knots for a 5-min period and the sampler was lowered 1 m each half-minute. Samples were collected from 1 to 10 m and 10 to 19 m.

Samples were diluted with 10% formalin to a volume between 100 and 800 ml depending on the settled volume of zooplankton (smallest volume diluted to 100 ml, largest to 800 ml). From these diluted samples, three 1-ml subsamples were taken and the total number of animals of each species counted. Means of the three subsamples were multiplied by the dilution factor to determine the total number of each species present. Kutkuhn (1958) found that adequate estimates of macroplankton species were obtained from counts of only three cell mounts per sample.

Sampling variability of the Wisconsin samples was determined by the coefficient of variability (Steel and Torrie, 1960, p. 20). It ranged from 0 to 24% with a mean of 6.0% for the total vertical hauls and 9.6% for hauls from 10 m to the surface. Sampling variability for Clarke–Bumpus samples was assessed by collecting five samples in rapid succession from the same station and depth. A test for homogeneity of variance using Bartlett's test (Steel and Torrie, 1960, p. 347) indicated no significant differences at the 5% level.

A test for homogeneity of variances between subsamples was conducted using the procedure of Ricker (1937). Eighty-three percent of the variances were not significantly different at the 5% level; therefore, subsampling variability was not considered to be excessive.

RESULTS

Macrozooplankton Changes between 1949 and 1964

The macrozooplankton of Kootenay Lake consists of four species of copepods, six cladocerans, and one mysid. Of these 11 species only six are common. The two most abundant forms are *Diaptomus ashlandi* Marsh and *Cyclops bicuspidatus thomasi* S. A. Forbes. Two other copepods, *Epischura nevadensis* Lilljeborg and *Diaptomus kenai* M. S. Wilson, are rare. Cladocerans are less abundant and are usually evident only in the summer and autumn. The two most abundant cladocerans are *Daphnia galeata mendotae* Birge and *Diaphanosoma leuchtenbergianum* Fischer. *Bosmina coregoni* Baird is also present. Extremely rare are *Scapholebris kingi* Sars, *Leptodora kindtii*, and *Ceriodaphnia* sp. *Mysis relicta* Lovén was introduced into the lake in 1949 (Sparrow et al., 1964).

All species present in 1949 were found in 1964 but were more abundant in 1964 (Fig. 2), both for total vertical hauls and for hauls from 10 m to the surface. June abundance did not differ significantly ($P = 0.05$) between 1949 and 1964. The greatest difference between the 2 years occurred in August. The mean number of animals per liter of all species of macrozooplankton for

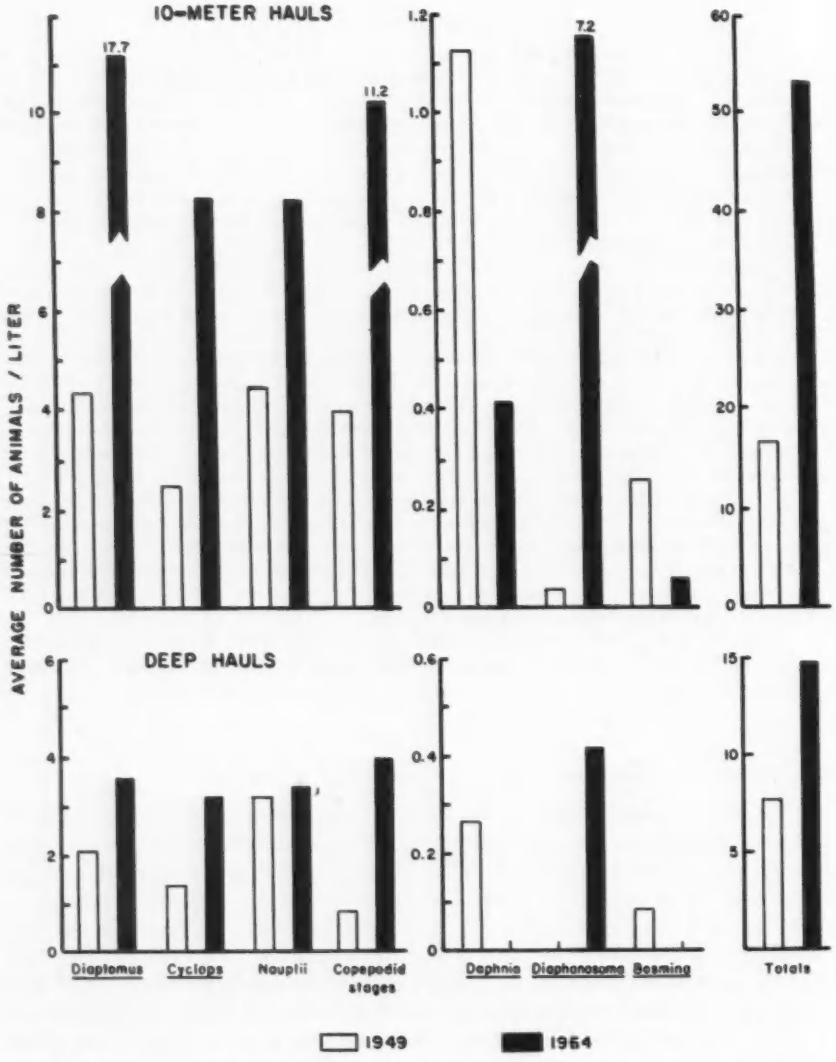

FIG. 2. Average number of animals at all stations during the summers of 1949 and 1964 (June to August) on Kootenay Lake.

stations throughout Kootenay Lake during the summer of 1949 and 1964 is as follows:

	Deep hauls		10-m hauls	
	1949	1964	1949	1964
June	7.81	8.85	16.07	20.17
July	7.47	13.23	13.84	26.17
Aug.	8.15	21.96	20.66	113.11
Total	23.43	44.04	50.57	159.45

The only species more numerous in 1949 than in 1964 were *Daphnia* and *Bosmina*. Although these species were not abundant in 1949, there was a marked decrease in their abundance in 1964 (Fig. 2). Other species increased sharply, particularly *Diaphanosoma* (Fig. 2).

The 0–10-m vertical zooplankton hauls indicated a greater concentration of zooplankton in the upper 10 m in 1964 than in 1949 (Fig. 2).

Daphnia was distributed throughout the lake in 1949 but was most abundant in the north arm (Table 1). In 1964 it was extremely rare in the north arm

TABLE 1. Mean number of *Daphnia* per liter for three areas of Kootenay Lake during the summers of 1949 and 1964.

	Deep hauls				10-m hauls					
	June		July		June		July		Aug.	
Area	1949	1964	1949	1964	1949	1964	1949	1964	1949	1964
North arm	0.50	0	0.53	0	–	–	0.76	0	2.57	0
Central area	0.06	0	0.13	0	0.44	0	0.80	0	2.17	2.48
South arm	0.24	0	0.31	0	0.56	0.01	0	0.03	–	–

but was present in the central area and south arm of the lake. *Daphnia* was relatively abundant during June and July 1949 but in 1964 it was not common until August.

CHANGES IN MIDSUMMER ZOOPLANKTON ABUNDANCE BETWEEN SUCCESSIVE YEARS

To validly compare between 1949 and 1964, samples were collected from the upper 19 m during July 1964, 1965, and 1966 to compare zooplankton abundance in 1964 with that in subsequent years. Total zooplankton was *least* abundant in 1964: the mean number of organisms per liter for all sampling sites was 11.33. Zooplankton was most abundant in 1965 (15.39 organisms/liter) and 1966 (14.44 organisms/liter). The sparse *Daphnia* and *Diaphanosoma* populations did not follow this pattern. The number of *Daphnia* per liter in the upper 19 m during July 1964, 1965, and 1966 was 0.11, 0.08, and 0.0 respectively. *Diaphanosoma* abundance was 0.33, 0.16, and 0.04 organisms/liter for the same years.

Physical–Chemical Limnology

Generally, Kootenay Lake is thermally stratified from late June to October and is isothermal for the remainder of the year. Temperatures below 50 m are near 4 C and undergo little change throughout the year (Zyblut, MS, 1967).

June, July, and August water temperatures were considerably colder in 1964 than in 1949 (Fig. 3). The exception occurred in August 1964 when water

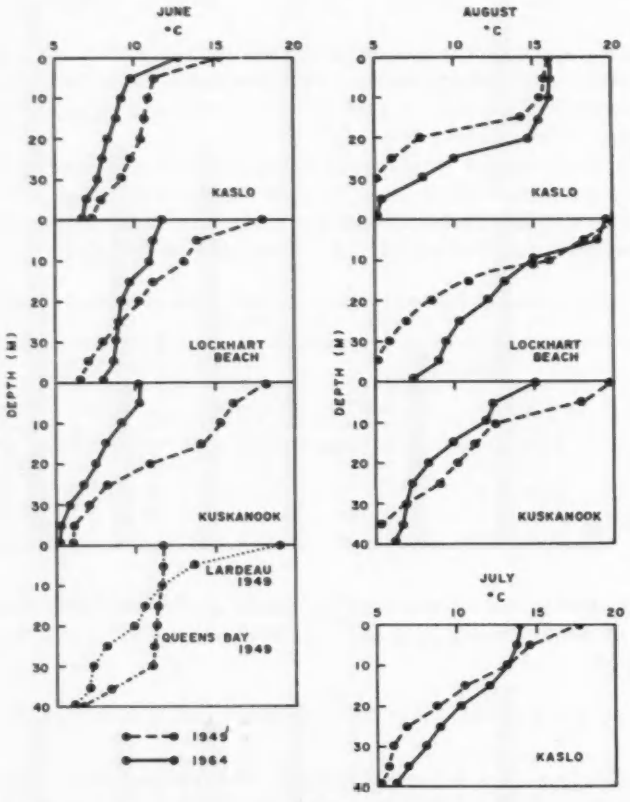

Fig. 3. Vertical temperature profiles for stations on Kootenay Lake during the summers of 1949 and 1964.

in the north arm was the warmer. Warmer lake temperatures in 1949 resulted from the earlier summer and warmer air temperatures that year (Anonymous, 1949, 1964).

The depth of light penetration was less during the summer of 1964 than 1949 (Fig. 4).

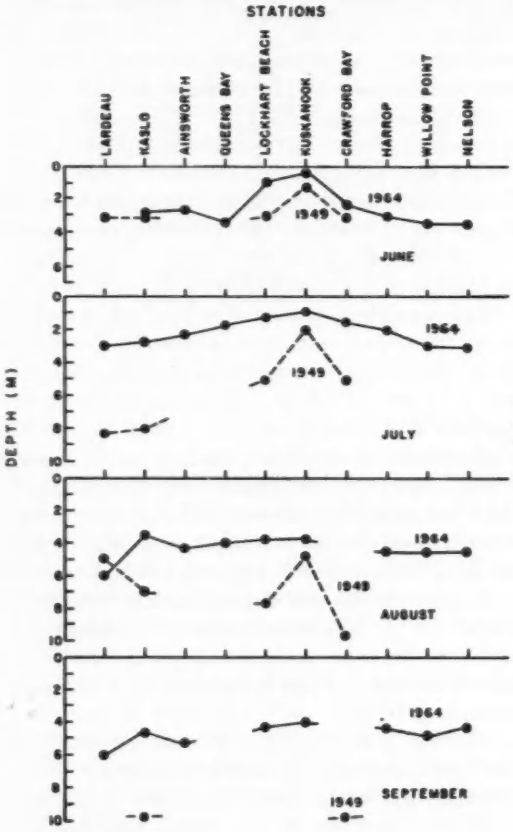

FIG. 4. Secchi disc measurements during the summers of 1949 and 1964 at representative Kootenay Lake stations.

Mean summer surface *p*H values were higher in 1964 than in 1949 in the west arm (8.16 cf. 7.95) and in the north arm (8.42 cf. 7.90) as was surface specific conductance, particularly in the west arm (161 μmhos/cm cf. 148) and in the north arm (127 cf. 125). Concentrations of several ions in the lake have changed since 1949; Mg, Cl, and SO_4 have increased by approximately 2 mg/liter and Ca has decreased by this amount.

Phosphate concentrations increased markedly since 1949 (Table 2). In the north arm an increase of 3–10-fold occurred although concentrations of phosphates in the Duncan and Lardeau rivers did not increase from 1949 to 1964. The phosphate concentration in the south arm increased 7–10-fold, and water entering the south arm via the Kootenay River had a larger concentration in 1964. Taylor (MS, 1968), in his study of the south arm of Kootenay Lake, found considerably higher phosphate concentrations than presented in this study.

TABLE 2. Phosphate phosphorus content in μg-at/liter during August 1949 and 1964. Data for 1949 obtained from C. Goldie and for 1964 from D. B. Fillion.

Station	Surface		Bottom	
	1949	1964	1949	1964
Duncan R.	0.095	0.072	*	*
Kaslo	0.035	0.168	0.042	0.414
Ainsworth	0.042	0.126	0.042	0.672
West arm near Balfour	0.063	0.216	*	*
Lockhart Beach	0.042	0.312	0.063	0.618
Kuskanook	0.063	0.594	0.053	0.498
Inlet of Kootenay R.	0.063	1.548	*	*

*No sample taken.

DISCUSSION

The summer zooplankton abundance in Kootenay Lake has increased markedly between 1949 and recent years (1964–66), indicating that the lake is becoming more productive and is undergoing accelerated eutrophication. Hasler (1947) referred to an increase in zooplankton in Zurichsee and attributed this to a gain in plant nutrients. Also Beeton (1965) suggested that increases in Lake Erie zooplankton were a result of accelerated eutrophication. Similarly, the increase in zooplankton abundance in Kootenay Lake may reasonably be attributed to eutrophication.

Further evidence suggesting that Kootenay Lake is undergoing accelerated eutrophication is discussed below.

Hasler (1947), Edmondson (1959), and Beeton (1965) found that an increase in eutrophication resulted in a decrease in lake transparency. Also, Nelson and Edmondson (1955) found that after annual fertilization of Bare Lake, the phytoplankton population increased and the Secchi disc transparency decreased from 6 to less than 2 m. Therefore, the large reduction in the depth of light penetration that occurred in Kootenay Lake since 1949 (Fig. 4) suggests an increase in phytoplankton.

The *p*H in Kootenay Lake being higher in 1964 than 1949 indicates that a larger phytoplankton population was present in 1964. In Lake Erie, which is undergoing accelerated eutrophication, Verduin (1964) found that before 1950 the *p*H was 8.7 and during 1960–61 it was 9.2. Similarly in Bare Lake, Nelson and Edmondson (1955) found that an increase in *p*H from 7.0 to 9.0 corresponded with an increase in phytoplankton. Likewise, Nelson (1966) found that higher *p*H values were recorded when phytoplankton populations were dense; and D. B. Fillion (personal communication) found a similar relation in Kootenay Lake.

Another indicator of eutrophication is an increase in nutrients. Beeton (1965) found that over the past 50 years ion concentrations in Lake Erie have increased. Similarly in Kootenay Lake the surface specific conductivity and

some ions have increased since 1949, suggesting that Kootenay Lake is becoming more eutrophic.

Abundance of phytoplankton is often limited by low concentrations of phosphate. In Kootenay Lake the concentration of phosphate has increased since 1949 (Table 2). Both Mortimer (1939) and Mackereth (1953) found that phosphate was conducive to greater phytoplankton abundance. Edmondson et al. (1956) found that in Lake Washington, where large blooms of algae and other changes associated with eutrophication have occurred, the maximum phosphorus concentration observed in the hypolimnion during 1955 was 1.24 μg-at/liter in contrast to a previous maximum of 0.71 μg-at/liter in 1933. The 1955 maximum is less than the phosphate concentration of water entering Kootenay Lake (Table 2). In Kootenay Lake, abundance of phytoplankton may have increased with the concentration of phosphate.

Changes indicated above suggest that Kootenay Lake is undergoing accelerated eutrophication. This may be from fertilization of agricultural land in the Kootenay Lake drainage area. According to Brink and Farstad (1949) there are approximately 25,000 acres of alluvium at the southern end of Kootenay Lake and 5000 acres of intensively farmed terrace. The application of fertilizer on this land has doubled since early 1950. Verduin (1964) found that phosphate concentration in western Lake Erie increased from 105 μg/liter in 1948 to 450 μg/liter in 1962, and attributed this to increased fertilization of farms in the Maumee River watershed.

The most important cause for the accelerated eutrophication in Kootenay Lake is a large fertilizer plant situated on a tributary of the Kootenay River (Fig. 1, inset). This plant started production in 1953, doubled its production in 1962, and tripled in 1964. However, in more recent years production has been reduced due to a decrease in world markets. Taylor (MS, 1968) states that this plant releases approximately 40–60 tons of phosphate material and 4–6 tons of nitrate daily and significantly contributes to the nutrient content of Kootenay Lake.

Although total zooplankton abundance has increased since 1949, both *Daphnia* and *Bosmina* were more numerous in 1949 (Fig. 2). Reduction of *Daphnia* may be greater than shown in Fig. 2 because *Daphnia* was more abundant in 1964 than in 1965 or 1966. Since 1949, *Diaphanosoma* has increased considerably and the average standing crop of zooplankton in Kootenay Lake has tripled. This trend is similar to that found by Bradshaw (1964) in Lake Erie where the average standing crop of plankton increased threefold over a 10-year period and *Daphnia pulex* decreased and *Diaphanosoma leuchtenbergianum* increased. Also, in some of the large lakes undergoing accelerated eutrophication, *Bosmina longirostris* was found to replace *B. coregoni* (Hasler, 1947; Beeton, 1965; Edmondson et al., 1956). In Kootenay Lake *B. coregoni* was the only species found during both years.

Changing limnological conditions may have altered the phytoplankton species since 1949. The reduction of *Daphnia* and increase in *Diaphanosoma* abundance may be related to the change in phytoplankton. Various authors

have found that *Daphnia* populations are correlated with food supply (Slobodkin, 1954; Hazelwood and Parker, 1961; and Wright, 1965). Burns (1968) found that *Daphnia* are selective feeders and there is a relation between the size of the animal (carapace length) and the size of the food ingested. Likewise, Brooks (1969) states that larger Cladocera feed on larger net phytoplankton. In Kootenay Lake it is possible that there has been a slight decrease in the size of the cells of the phytoplankton community. *Diaphanosoma*, because it is smaller than *Daphnia*, may be more efficient at utilizing this food source and hence has increased in abundance.

Brooks (1969) states that planktivorous fish are highly selective in their choice of food items, preferring the larger zooplankton, and have a preference for cladocerans over copepods of the same size. When size-dependent predation is intense this will eliminate larger forms and allow the small zooplankton to become the dominants. Likewise, Galbraith (1967) found that only the *Daphnia* over 1.3 mm in size were consumed by rainbow trout and yellow perch and the more numerous smaller zooplankton were ignored. There is a large population of planktivorous kokanee (*Oncorhynchus nerka*) present in Kootenay Lake. Juvenile kokanee are known to selectively feed on *Daphnia* (R. D. Beauchamp, personal communication). Unfortunately data are not available to determine if the kokanee population has increased in recent years. However, even if there has not been an increase, the selective feeding by kokanee on the larger *Daphnia* and the change in food size available may account for a reduced *Daphnia* population.

Changes in spatial distribution of zooplankton between 1949 and 1964 was exhibited only by *Daphnia*. This species was present in large numbers in the north arm during the summer of 1949 but not in 1964 (Table 1). Recent sampling indicates that *Daphnia* appear in the north arm for approximately 1 month, usually in September. The change in distribution may be a result of size-selective predation and competition. Both Brooks (1969) and Burns (1969) feel that zooplankton distribution and composition can be changed by these factors. Brooks (1969) points out that this caused a change in Lake Michigan zooplankton. In Kootenay Lake, the area with the greatest abundance of juvenile kokanee is in the north arm (A. H. Acara, personal communication). Hence predation and changes in food may restrict the abundance of *Daphnia* in the north arm. Futhermore, the introduction of *Mysis relicta*, which is most abundant in the north arm of the lake (Zyblut, MS, 1967), may restrict *Daphnia* through competition for food. Owing to the larger body size *M. relicta* would be more efficient than *Daphnia* in filtering the larger phytoplankton, and *Diaphanosoma* may be more efficient at filtering the smaller food. Frank (1952), studying intraspecific and interspecific competition in *Daphnia pulicaria* and *Simocephalus vetulus*, found that in single species populations, neither species ever approached extinction. However, in mixed species populations, *Simocephalus* invariably died out and *Daphnia* assumed the characteristic of a single species culture.

D. B. Fillion (personal communication) found that in Kootenay Lake a large phytoplankton bloom occurs during late summer in the north arm. Therefore food may be abundant in the north arm, permitting *Daphnia* to coexist with *Diaphanosoma* and *M. relicta*. Pejler (1962) states that zooplankton may avoid competition during a temporary superabundance of food, which enables forms feeding on the same matter to increase simultaneously.

The reduction in size of the *Bosmina* population since 1949 may be a result of the increase in abundance of *Diaphanosoma*. This species is larger than *Bosmina* and is more efficient in filtering food and consequently may be eliminating *Bosmina* through competition. Brooks (1969) found that due to a decrease in predation of coregonids on *Daphnia galeata mendotae* in Lake Michigan, this species, through competition, eliminated *Bosmina*.

All changes in limnological conditions and zooplankton distribution and abundance suggest that Kootenay Lake is undergoing accelerated eutrophication. Although most of the changes are not spectacular, they have occurred in 15 years. During the next 25 years conditions around Kootenay Lake will favour further eutrophication (increase in population and more industrial developments). Consequently, it is not only the shallow, relatively warm lakes but also cold, deep oligotrophic lakes that can be subjected to accelerated eutrophication.

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Levels of Production in the Pelagic Environment of the Strait of Georgia, British Columbia: A Review

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PARSONS, T. R., R. J. LEBRASSEUR, AND W. E. BARRACLOUGH. 1970. Levels of production in the pelagic environment of the Strait of Georgia, British Columbia: a review. J. Fish. Res. Bd. Canada 27: 1251–1264.

Data have been accumulated on seasonal levels of nutrients, primary production, and zooplankton. The occurrence and abundance of larval fish in the surface layers have been reported together with an approximate estimate of the standing stock of commercially exploited fish. The results indicate that beyond local effects, the Strait of Georgia is comparable in productivity to other nearshore waters at the same latitude. The total primary productivity of the waters was found to be approximately 120 g C/m² per year, but a high degree of areal patchiness in all production data emphasizes the necessity of basing predictions about particular subareas on knowledge specific to the subareas in question.

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INTRODUCTION

THIS general description of biological production in the Strait of Georgia summarizes a large amount of data that was collected as part of Canada's contribution to the International Biological Program. It is intended to serve for comparing the productivity of the Strait of Georgia with other waters of national and international interest to Canada and as a basis for evaluating subsequent changes in productivity, such as might be caused by sustained pollution of this environment. The primary intention of this report is to describe levels of production rather than to discuss detailed relations between producers; some primary publications have already appeared dealing with particular aspects of the latter (e.g., Parsons and LeBrasseur, 1970; LeBrasseur, 1969; Parsons and Seki, 1970). The original source for data reported here and in the references cited above comes from the following data and technical reports: Bishop et al. (MS, 1966), Fulton et al. (MS, 1967, 1968, 1969a, b), Stephens et al. (MS, 1969), Stephens (MS, 1968), Barraclough (MS, 1967a, b, c), Barraclough and Fulton (MS, 1967), Barraclough et al. (MS, 1968), Robinson et al. (MS, 1968), and Robinson (MS, 1969a, b). Some previously unreported data on the occurrence of larval and juvenile fish, outside of the Fraser River plume, have also been included. Data on adult fish stocks were taken from previous reports that were not immediately associated with the program reported here. These data were included to provide some appreciation of the adult fish stocks of the area for purposes of comparison with other environments of possible interest to fisheries. Three estimates of production

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omitted from this report are the primary production of macrophytes, littoral production, and benthic production; no appreciable data were collected on these subjects during the study period.

METHODS

Methods for the collection and analysis of samples are described in the Fisheries Research Board of Canada reports (mentioned above) covering studies in the Strait of Georgia, 1965–68. Generally, collections of nutrients, plankton, and larval and juvenile fish were made at 10–15 stations during cruises covering the entire Strait. Four or five cruises were carried out each year; in addition, supplementary observations were made at a central location in the Strait, as well as in inlets and in the estuaries of the Fraser and Nanaimo rivers. The distribution of principal sampling stations is shown in Fig. 1.

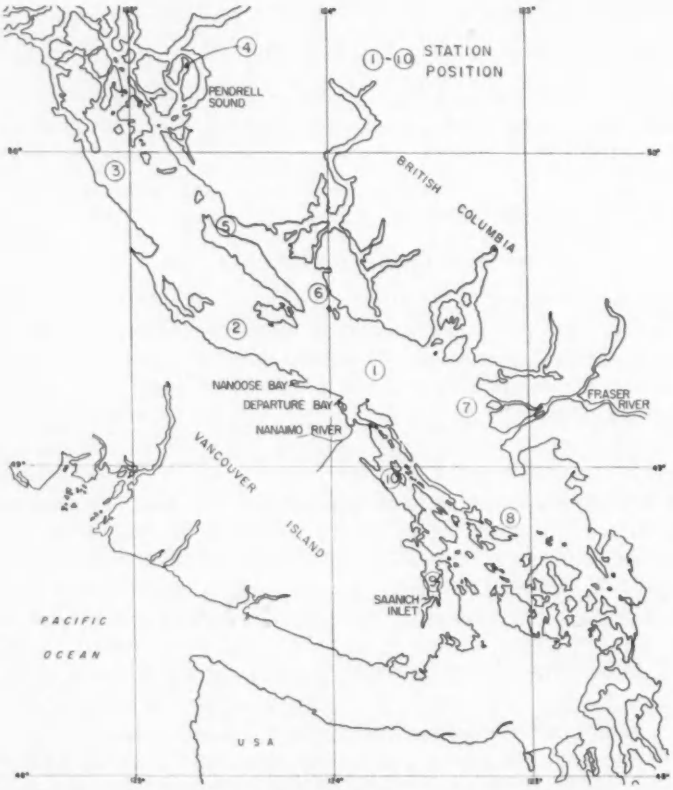

FIG. 1. Routine sampling stations in the Strait of Georgia.

The productivity data and zooplankton standing stock have been summarized (Table 1). The mean, standard deviation from the mean, and the maximum and minimum values were calculated for the nutrient and primary productivity data from observations collected from 1965 through 1967. The standard deviation for zooplankton concentrations was determined sepa-

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Max	Min
Irradiance (*ly/month*)[a]	75 (9)	166 (34)	273 (57)	412 (30)	562 (54)	599 (122)	606 (37)	503 (71)	362 (29)	171 (19)	95 (14)	60 (5)	701	57
Secchi depth (*m*)	9.5 (3.0)	8.5 (1.7)	9.5 (3.2)	7.5 (3.8)	4.0 (2.9)	3.8 (2.9)	5.0 (4.8)	5.8 (2.6)	11.6 (2.6)	8.2 (2.6)	8.4 (2.0)	9.3 (6.3)	16	1
Silicate (*μg-at/liter*)[b]	[c]	56 (5)	50 (13)	22 (10)	48 (20)	33 (9)	24 (10)	25 (15)	26 (11)	38 (13)	44 (4)	52 (4)	100	8
Nitrate (*μg-at/liter*)[b]	22 (0.8)	23 (0.8)	21 (2.7)	15 (3.3)	14 (2.9)	7 (2.8)	8 (4.2)	9 (4.4)	12 (4.9)	17 (6.0)	23 (2.3)	24 (1.9)	27	< 1
Phosphate (*μg-at/liter*)[b]	2.0 (.18)	2.1 (.19)	1.8 (.29)	1.6 (.69)	1.5 (.32)	0.9 (.41)	0.8 (.29)	0.9 (.37)	1.1 (.48)	1.4 (.41)	1.9 (.23)	2.3 (.53)	3.2	0.3
Seston (*mg/liter*)[b]	3.3 (2.6)	1.0 (0.7)	2.0 (2.1)	2.5 (1.6)	4.2 (3.0)	4.8 (2.6)	2.7 (1.4)	2.7 (1.7)	1.8 (1.0)	2.0 (1.7)	1.8 (1.3)	[c]	8.8	0.2
Particulate C (*μg/liter*)[b]	250 (64)	200 (57)	210 (57)	220 (110)	320 (130)	410 (95)	390 (130)	410 (160)	280 (130)	230 (86)	170 (43)	180 (87)	740	50
Particulate N (*μg/liter*)[b]	15 (3.4)	16 (13)	21 (5.3)	32 (11)	32 (26)	53 (13)	50 (17)	40 (19)	34 (15)	32 (11)	15 (2.9)	14 (5.9)	115	9
Chlorophyll *a* (*μg/liter*)[b]	0.9 (0.3)	1.0 (0.5)	2.1 (1.6)	3.0 (2.1)	3.2 (2.4)	4.3 (1.9)	3.0 (1.6)	2.4 (1.4)	2.0 (1.1)	2.0 (1.3)	0.8 (0.5)	1.0 (0.4)	10	< 0.5
Soluble C (*mg/liter*)[b]	[c]	1.5 (0.6)	2.0 (1.1)	2.5 (1.1)	2.8 (0.9)	2.9 (1.4)	3.0 (1.9)	3.2 (1.9)	3.0 (1.0)	2.0 (1.0)	1.2 (0.1)	1.2 [c]	8.5	0.3
Primary productivity (*mg C/m² per day*)	20 [c]	250 (122)	470 (240)	550 (450)	1200 (820)	320 (300)	270 (130)	310 (280)	200 (120)	230 (85)	120 (48)	[c]	2562	10
Total herbivorous zooplankton[d] (*mg wet wt/m³*)	50	60	450	950	1420	700	300	120	100	80	50	50	1750	< 50
Principal zooplankton species[e]														
Herbivorous zooplankton														
Calanus plumchrus (*mg wet wt/m³*)	10	28	2600	4280	2800	320	50	10	0	0	0	0	6000	0
Calanus pacificus (*mg wet wt/m³*)	[c]	24	46	94	86	44	38	280	320	70	14	6	1000	0
Pseudocalanus minutus (*mg wet wt/m³*)	[c]	2	20	26	20	18	22	14	14	26	10	4	60	0
Carnivorous zooplankton														
Pleurobrachia and *Philidium* (*no./m³*)	–[f]	–	–	–	–	7	70	63	13	3	–	–	120	0
Sagitta elegans[g] (*no./m³*)	[c]	[c]	0.8	2.7	[c]	[c]	3.4	6.0	[c]	1.5	[c]	[c]	[c]	[c]

[a] Measured at Departure Bay.
[b] Average value for 0–20 m.
[c] Insufficient data to report a value.
[d] 1965–67 average value for 20-m vertical net haul at eight stations using a 350-μ Hensen net.
[e] 1966–68 average for station 1, 20-m vertical haul using a 350-μ Hensen net.
[f] Dash represents organisms not found in 20-m collections.
[g] Data available for only 5 months in 1965; tows made from 100 m to the surface.

rately from areal surveys (Stephens et al., MS, 1969) and shown to vary from 30 to nearly 100% of the mean. It is not the purpose of this report to discuss factors contributing to variability in the data. However, factors such as depth, tide, water mass, and location of sampling stations, which contribute to variability in production data, can often be brought together to account for a particular biological event (e.g., the spring phytoplankton bloom, Parsons et al., 1969b). In this report the range of productivity values for the whole area has been presented to show seasonal trends that are independent of local variations in the data.

RESULTS

Nutrients, Primary Production, and Particulate Material

Conditions governing the onset of the spring phytoplankton bloom in the Strait of Georgia were discussed by Parsons et al. (1969b). The principal conditions are the increase in solar radiation during the period February to April and the formation of a shallow mixed layer. The bloom occurs first in the vicinity of the Fraser River plume and later over other parts of the Strait. A decrease in plant nutrients can first be detected in March and a large increase in the ^{14}C-primary production of the water column occurs from January through May (Table 1). A pronounced decrease in primary production from June through August is accompanied by a marked drop in nutrients in the upper 20 m. The average standing stock of phytoplankton in the first 20 m (chlorophyll *a*) reaches a maximum during June; the maximum in primary production occurs in May. The total annual primary productivity of the Strait is approximately 120 g C/m^2 per year, but in some inlets the production may be greater (Stephens et al., 1967) because of the effects of nearshore tidal mixing, which partially restores nutrients to the euphotic zone throughout the summer.

Variations with depth in the distribution of chlorophyll *a*, primary productivity, and nitrate were taken as examples from Stephens et al. (MS, 1969) (Fig. 2). The standing stock and primary production of phytoplankton are maximal at or very near the surface during the autumn, winter, and spring, but maximum primary production occurs below the surface after nutrient depletion during the summer.

In addition to the production of particulate carbon by primary producers, there is also a continual transport of allochthonous particulate carbon into the Strait from land drainage. In quantity this has been estimated to be at least as great as the total annual primary production (Stephens et al., 1967; Seki et al., 1969). However, the utilization of this material, through a bacterial cycle, and back to filter feeders is only, at the most, 30% as efficient as the direct utilization of particulate carbon produced by phytoplankton (Seki et al., 1968). Maximum levels in soluble carbon (Table 1) occur in late summer, and this is probably due to a partial senescence of living material in the upper 20 m, but it may also be associated with attached macrophyte production as discussed by Sieburth and Jensen (1969).

Herbivorous and Carnivorous Zooplankton

The total average standing stock of herbivorous zooplankton in the first 20 m (Table 1) reaches a maximum over the whole Strait during May. This

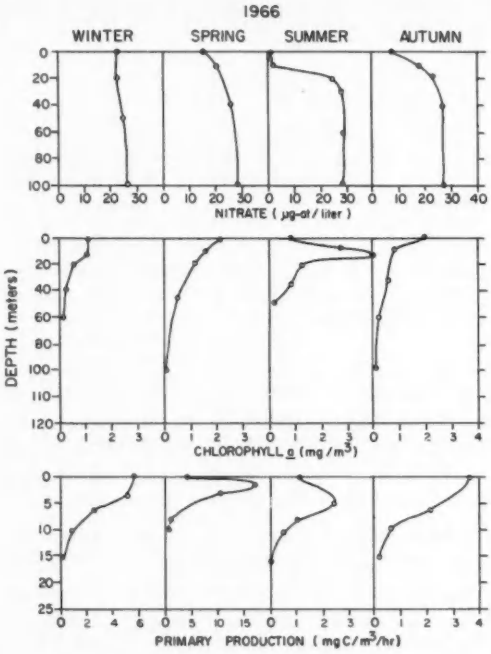

FIG. 2. Seasonal changes in the depth distribution of nitrate, chlorophyll *a*, and primary production in the Strait of Georgia (data from 1966).

event is primarily determined by one species of copepod, *Calanus plumchrus*. The annual production of this animal, particularly in reference to its occurrence in dense aggregations off the Fraser River estuary, has been described by Parsons et al. (1969a). *Calanus plumchrus* nauplii first occur in the water column during February; the animal then develops in the near surface waters from copepodite stages I through V during a 3-month period, March through May, after which it descends to approximately 300 m, where it matures to stage VI without feeding; eggs are released at depth during January and February of the following year.

Although there are many other herbivorous zooplankton in the Strait, both strictly planktonic species and holoplanktonic species (e.g., barnacle larvae), the only other species that consistently contribute significantly to the total zooplankton biomass are *Calanus pacificus* and *Pseudocalanus minutus*. Both of these species have more than one generation per year and show bimodal peaks in biomass production (Table 1).

The smaller microzooplankton (250–512 μ diameter) do not contribute significantly to the total biomass of zooplankton, but, because of their importance as food for larval fish (LeBrasseur et al., 1969), they have been reported separately (Fig. 3). Their numbers ranged from <10 to 150/liter and generally consisted of the eggs and naupliar stages of copepods and euphausiids as well as adult stages of small copepods such as *Oithona spinirostris*

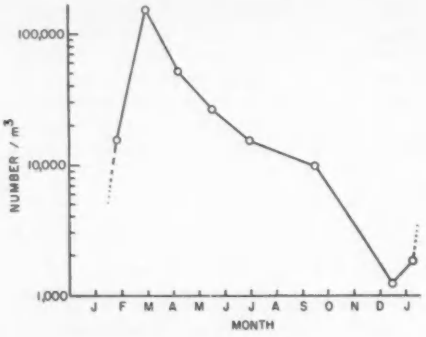

FIG. 3. Seasonal changes in the numbers of microzooplankton (250–512 μ diameter) in the Strait of Georgia (data from 1967).

and *Microcalanus*. The seasonal abundance of these organisms occurred as a sharp maximum in March, and from March through May there were at all times at least 20 organisms/liter (Fig. 3). According to Blaxter (1965) the value of 20 organisms/liter may be a critical level below which larval survival is severely affected.

Carnivorous zooplankton have been reported in Table 1 as number per cubic meter since these species are largely comprised of water and their weight is misleading in terms of actual organic material. However, dense swarms of *Pleurobrachia* and *Philidium* may occur during July and August. Data on *Sagitta elegans* would have been better represented by night tows, since the animal shows considerable diurnal migration (Fulton et al., MS, 1969a). However, apparently this animal also occurs in maximum numbers in the surface waters during the latter part of the summer.

FISH

An approximate assessment of total adult resident fish stocks fished commercially in the Strait of Georgia is shown in Table 2. The table does not include estimates of the stocks of larvae and juveniles of the species shown, nor is there any estimate of smelts (Osmeridae) and sand lance (*Ammodytes hexapterus*), two species of fish for which we have no comparable data. The estimates are regarded as minimal; the total fish stocks in the Strait are likely to be at least double the total stock of the commercially exploited fish. In addition, some juvenile species of salmon are numerically abundant as transients for periods of 6–10 weeks. As an indication of these numbers, the average annual output of juvenile salmon from the Fraser River alone could be estimated as 97×10^6 sockeye (*Oncorhynchus nerka*), 234×10^6 pink (*Oncorhynchus gorbuscha*), and 70×10^6 chum salmon (*Oncorhynchus keta*) (data from International Pacific Salmon Fisheries Commission 1960–63, and 1967; Todd, MS, 1965; and Larkin and Ricker, 1964). An equivalent number of juvenile pink and chum salmon may also enter the Strait from the surrounding tributary streams and rivers.

TABLE 2. Estimates of the total biomass of resident fishes in the Strait of Georgia that are harvested by the commercial fishery.

Species	Wt (*Tons T* × *10*$^{-3}$)	Reference
Herring (*Clupea pallasi*)	71	Larkin and Ricker, 1964
Lingcod (*Ophiodon elongatus*)	18	Larkin and Ricker, 1964
Cod (Gadidae)[a]	3–6	Prest, MS, 1967
Flatfish (Pleuronectidae)[a]	2–4	"
Rockfish (Scorpaenidae)[a]	0.5–1	"
Dogfish (*Squalus suckleyi*)[b]	20	"
Coho salmon (*Oncorhynchus kisutch*)[c]	1.3	Dep. of Fish., 1962
Chinook salmon (*Oncorhynchus tshawytscha*)[c]	.8	Dep. of Fish., 1962
Miscellaneous[a,d]	2–4	Prest, MS, 1967
Total	118–126	

[a]Catch figures (Prest, MS, 1967) were determined according to the method suggested by Alverson and Pereyra (1969) for the total stock estimates.
[b]Stock estimates for dogfish were adjusted from figures given by Prest (MS, 1967) and Shepard and Stevenson (1956).
[c]Catch statistics for coho and chinook salmon exclusive of the Fraser River were doubled to include escapement.
[d]Included in the miscellaneous species are sablefish, perch, sturgeon, shad, whiting, arrowtooth flounder, squid, octopus, and skate.

The occurrence of larval and anadromous juvenile fish is shown in Table 3 for the surface waters. Most larvae are present during the first 6 months of the year and there is a general absence of fish larvae from October through December. The only notable exception to this are larval capelin (*Mallotus villosus*). Juvenile anadromous fishes first occur in the Strait during the period March to July, whereas juveniles of marine species are found in the latter part of the year. The maximum number of larvae occur in the period March–April, and the diversity of larval species increases from May through July (Table 4).

AREAL DISTRIBUTIONS

PHYTOPLANKTON AND PARTICULATE MATERIAL

The areal distribution of chlorophyll *a* and particulate materials as measured with a continuous chlorophyll *a* analyser and turbidity meter has been illustrated by Stephens (MS, 1968). Generally during the spring, summer, and autumn, large differences in chlorophyll *a* concentration are encountered in the vicinity of the Fraser River plume, with less variation in the northern parts of the Strait (e.g., Fig. 4). The former variations are associated with changes in water masses in the immediate area of the Fraser River plume, and as such they are subject to considerable lateral movement during a single tidal cycle. The distribution of a few individual phytoplankton species causing blooms has been shown at times to cover the entire Strait and at other times to be characteristic of a single station (Stephens et al., MS, 1969). Generally

TABLE 3. Occurrence of larval (L) and anadromous juvenile (J) fish in the Strait of Georgia.

Species caught	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Lampetra ayresi				J	J	J						
Clupea pallasi			L	L	L	L	L					
Engraulis mordax						J	J					
Oncorhynchus sp.[a]				J	J	J	J					
Thaleichthys pacificus			L	L	L	L	L					
Hypomesus pretiosus							L	L	L			
Mallotus villosus	L	L								L	L	L
Leuroglossus stilbius	L	L	L	L	L							
Merluccius productus			L	L	L	L	L					
Theragra chalcogrammus		L	L	L	L	L						
Pleuronectidae[b]			L	L	L	L	L	L	L			
Hexagrammos decagrammus	L	L	L	L	L							
Hexagrammos stelleri	L	L	L	L	L							
Ophiodon elongatus	L	L	L	L	L							
Sebastodes sp.				L	L	L	L					
Scorpaenichthys marmoratus		L	L	L	L	L						
Hemilepidotus hemilepidotus		L	L	L	L	L						
Gilbertidia sigalutes		L	L	L	L	L						
Agonopsis emmelane				L	L	L						
Gasterosteus aculeatus			J	J	J	J	J					
Syngnathus griseolineatus							L	L	L			
Coryphopterus nicholsii						L	L					
Bathymaster signatus			L	L								
Stichaeidae	L	L	L	L	L	L	L					
Lumpenus sagitta			L	L	L							
Lyconectes aleutensis		L	L	L	L	L						
Ammodytes hexapterus (larval)		L	L	L	L	L						

[a]Including all five species of juvenile salmon.
[b]Including *Platichthys stellatus*, *Hippoglossoides elassodon*, *Isopsetta isolepis*, and *Lyopsetta exilis*.

TABLE 4. Catches of larval fish in net tows in Strait of Georgia, 1966–67.

	No. tows	Vol water sampled ($m^3 \times 10^{-3}$)	No. larvae per 1000 m^3		No. species present
			Mean	Range	
Jan.	–	0	–	–	–
Feb.	41	45.3	1.0	0–100	7
Mar.–Apr.	52	44.5	7.0	0–1700	13
May–June	104	151.0	3.7	0–1300	14
July	74	108.8	0.8	0–7	9
Aug.	–	0	–	–	–
Sept.	58	42.5	1.4	0–166	3
Oct.	–	0	–	–	–
Nov.	17	88.5	0	0	0
Dec.	–	0	–	–	–

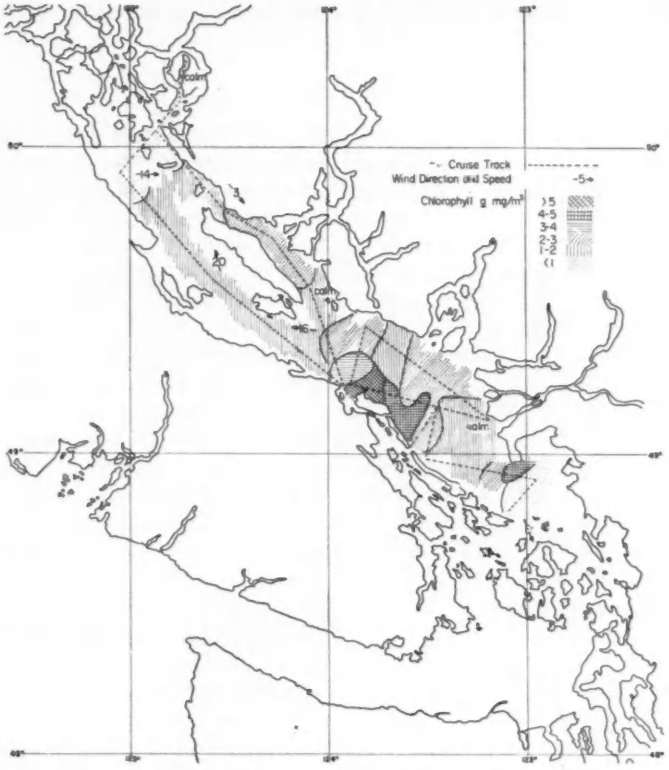

FIG. 4. Chlorophyll *a* distribution in the Strait of Georgia, March 23–29, 1966.

however, the phytoplankton is dominated during the spring and early summer by the presence of *Skeletonema costatum* with prominent occurrences of *Thalassiosira* sp., *Chaetoceros* spp., *Coscinodiscus* spp., and other genera of large diatoms; during the latter part of the summer dinoflagellates may occur in appreciable numbers. During the winter nanoplankton predominate, especially small flagellates, which may often form large blooms in early spring. One of the most common organisms causing a so-called "red tide" is the ciliate *Mesodinium rubrum* (Herlinveaux et al., MS, 1966; Parsons and Blackbourn, 1968), but toxic blooms of red tide also occur sporadically, and these are probably due to *Gonyaulax acatenella* (for a recent review, see Quayle, 1969).

The total load of particulate material is greatest in the vicinity of the Fraser River estuary during the annual freshet (May–June). Coastal areas are also subject to a heavy load of particulate material after the first heavy rainfalls of winter (Stephens et al., 1967). Particles carried by rivers during the winter are generally small in size (<4 μ diameter); larger particles (>10 μ)

generally predominate during the summer, both in rivers entering the Strait and in the sea (Seki et al., 1969).

Zooplankton

The areal distribution of zooplankton is predominated by the distribution of *C. plumchrus* during the period March–May. This organism occurs in very dense swarms in the vicinity of the Fraser River plume (Fig. 5), where

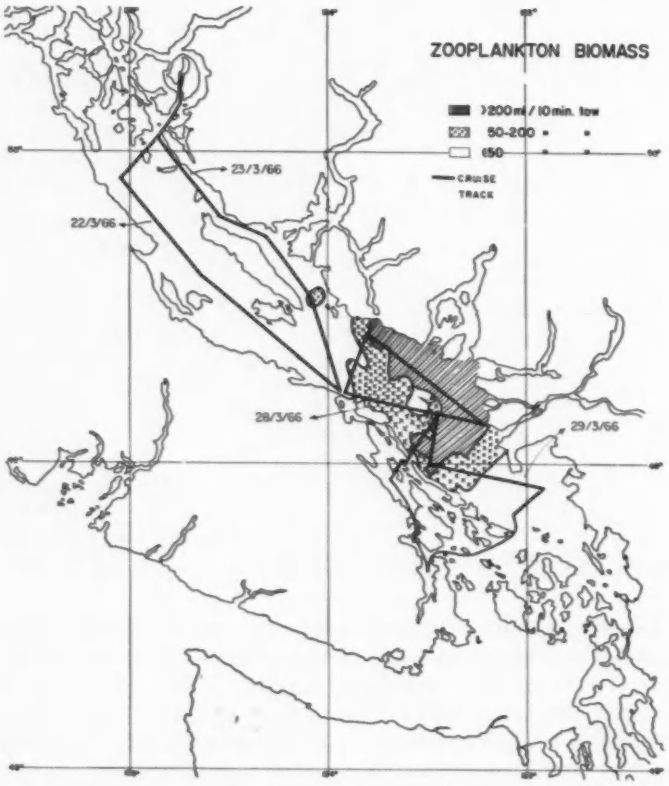

Fig. 5. Zooplankton distribution in the Strait of Georgia, March 23–29, 1966.

a net increase in primary production is maintained throughout the early spring months (Parsons et al., 1969 a, b). Within this area a high degree of local patchiness can result in a change of an order of magnitude in zooplankton concentrations within a distance of 90 m (Stephens et al., MS, 1969). During the summer and autumn a less intense areal variation in zooplankton concentrations is maintained because of local oceanographic conditions. With the onset of winter mixing, the zooplankton standing stock of the surface layers is low

and fairly uniform throughout the Strait, but large concentrations of zooplankton (e.g., *C. plumchrus*) can be found in deeper waters at about 300 m.

DISCUSSION

Previous reports on data relevant to the productivity of the Strait of Georgia have dealt with several separate parts of the environment, such as nutrients (Tully and Dodimead, 1957), plankton (Hutchinson and Lucas, 1931; Campbell, 1934), and the phosphorus cycle (Strickland and Austin, 1960). In this paper we bring together descriptions of the principal components in the various levels of pelagic production in the Strait of Georgia. The large amount of variability in the data (e.g., data in Fig. 4, 5) reflects the high degree of patchiness in the waters of the Strait of Georgia. Although the standard deviation is generally considered in a biometric sense as a measure of variation of the mean, the same statistic is used by Ivlev (1961) to reflect aggregation. As such, Ivlev's ζ (an index of aggregation) corresponds to σ, as used in Table 1. In the former sense, however, it is employed to assess the availability of prey, such as plankton, which is more readily obtained by a predator if its distribution is patchy (i.e., large ζ).

The primary production of the Strait of Georgia (120 g C/m^2 per year) is comparable to the continental slope off New York (120 g C/m^2 per year, Ryther and Yentsch, 1958) and to inshore Danish waters (97 g C/m^2 per year, Steemann Nielson, 1958, 1965). It is appreciably higher than oceanic production in the subarctic northeast Pacific (43–78 g C/m^2 per year, Anderson, 1964, for summary of values) but lower than Long Island Sound (180 g C/m^2 per year, Riley, 1956) and certain inlets around the Strait (e.g., Departure Bay, 200 g C/m^2 per year, Stephens et al., 1967) or on the Atlantic coast (e.g., St. Margaret's Bay, 193 g C/m^2 per year, T. Platt, personal communication). Apparently the open waters of the Strait of Georgia are at present not receiving any appreciable nutrient enrichment from agricultural lands or domestic outfalls.

During the spring zooplankton bloom in the Fraser River estuary (Parsons et al., 1969a), approximately 80 g wet wt/m^2 of *C. plumchrus* is produced, or about 4 g zooplankton C/m^2. During the same period the primary production was estimated to be approximately 50 g C/m^2 (Parsons et al., 1969b), which represents about half the annual primary production for the area. The annual production at these two levels is sustained by the stability of the water mass in the vicinity of the Fraser River plume, and by the increased solar radiation, March–May. Among factors that could eventually affect the magnitude and form of the production would be a decrease in water transparency or a substantial decrease in river flow.

The production of large diatoms in the Strait of Georgia appears (Parsons and LeBrasseur, 1970) to be a favorable event for the support of large copepods, which in turn are a highly suitable food for juvenile fish, such as young salmonids and herring. Studies in the Fraser River plume (LeBrasseur et al., 1969) showed that young salmon required prey densities of three or four copepods

per liter. Although these concentrations were present in the estuary, generally the distribution of zooplankton would have to be highly contagious (large ζ) for juvenile fish to obtain a sufficient food supply. A second food chain depending on the primary production of nanoplankton and the smaller microzooplankton appears to be restricted to a low level of productivity during the winter with large sporadic increments in nanoplankton, and consequent blooms of small microzooplankton, occurring for only short periods during the spring (Fig. 3). Thus, larval fish, shown (LeBrasseur et al., 1969) to feed primarily off zooplankton in the size category 250–512 μ, would be greatly influenced in their survival by variations in the quantity of the smaller microzooplankton; an effect that would be particularly critical in the period February–March when the number of microzooplankton increases from suboptimal concentrations for larval survival (<20/liter) to mean values of 150/liter. Fish stocks, such as herring and lingcod, which have pelagic larvae and are commercially important in the Strait (Table 2), could be highly susceptible to annual fluctuations in the environment of the Strait of Georgia, insofar as the survival of such larvae may depend on the type and concentration of their food supply (e.g., Rosenthal and Hempel, 1970). The most critical months for larval survival are from February to May (Table 3, 4).

In conclusion, at the time of the study, and beyond local effects, there was no apparent influence of pollution on the pelagic production of the Strait of Georgia. However, natural environmental conditions could influence the survival of larval fish and, to a lesser extent, juvenile anadromous fish.

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Growth and Reproduction of the Lanternfish *Stenobrachius leucopsarus*

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Growth and reproductive patterns of the common lanternfish *Stenobrachius leucopsarus* (Eigenmann and Eigenmann) are described by length-frequency analysis, otolith analysis, and examination of ovaries. Length-frequency analysis showed that growth is approximately linear, 1.59 mm standard length per month, during the 2nd, 3rd, and part of the 4th year of life. Yearling fish average 23 mm long, 2-year-olds 41 mm, and 3-year-olds 59 mm.

Otolith analyses indicate that some fish may live to be 8 years old, but confidence in this method is limited to fish 5 years old or younger. Fitting mean lengths of age-groups defined by otolith analyses with the von Bertalanffy equation gave $L_\infty = 85$ mm, $k = 0.34$. Back calculation of lengths at the times of formation of otolith annuli gave another set of estimated mean lengths of age-groups, which, fitted by the von Bertalanffy equation, describes a growth curve similar to the one described by otolith analyses. The inflection in growth in weight occurs at about 4 years of age.

Time of spawning, determined from egg measurements, occurs from December to March. Reproductively mature individuals are 4 years old and older. Recruitment of young size groups is also seasonal, 20- to 25-mm individuals appearing in largest proportions in trawl samples in the winter, presumably about 8 months after spawning.

Comparison with other studies indicates that spawning may occur earlier in Monterey Bay, California, than off Oregon, but growth rates and sizes of age-groups I–V are similar. Comparisons with published results of otolith analyses show similar age determinations for the smaller size groups.

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INTRODUCTION

STENOBRACHIUS LEUCOPSARUS is an abundant mesopelagic myctophid fish in the North Pacific Ocean. Aron (1962) reported it as the most abundant and most frequently caught fish in midwater trawl samples north of 45°N in the Pacific. Its zoogeographic range in the North Pacific extends north of about 35°N into the Gulf of Alaska and the Bering Sea (Parin, 1961). Paxton (1967) called the species Transitional-Subarctic in its water mass associations and presented further evidence for correlating its distribution with subarctic water.

The vertical distribution of *S. leucopsarus* is affected by the vertical stratification of water masses (Paxton, 1967; Aron, 1962), its ontogenetic development, and its vertical migrations. Fast (1960) and Ahlstrom (1959) reported that *S. leucopsarus* larvae are concentrated in the upper 100 m, localized particularly around 30–40 m. Fast's daytime observations in Monterey Bay, California,

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suggest that metamorphosis takes place at 400–500 m, juveniles rise to 200–300 m, and the fish gradually seek lower levels with increasing age. Taylor (1968) also indicated that during daytime, large individuals may occupy deeper levels than small individuals (455–595 vs. 230–275 m). During vertical migration, *S. leucopsarus* has also been associated with vertically migrating sound scattering layers (Taylor, 1968; Barham, 1957).

This paper describes growth, age, and season of spawning for *S. leucopsarus* off Oregon. Such knowledge is important to an understanding of the mortality, productivity, and ecology of a population.

Some information is available concerning age and growth of *S. leucopsarus*, but comprehensive data have not been published. Bolin (1956) and Fast (1960) determined growth by examining length frequency distributions from samples taken in Monterey Bay, California. Fast described a protracted spawning period from occurrence of larvae and mature females in these samples. Kulikova (1957) described growth by analyses of otoliths from 46 specimens collected in the Bering and Okhotsk seas and the Kuril–Kamchatka trench.

METHODS

Sampling

Fish used in this study were collected with a 6-ft Isaacs-Kidd midwater trawl (Isaacs and Kidd, MS, 1953; Aron, 1962) equipped with a 5-mm mesh liner and a 0.57-mm mesh codend. The trawl was equipped with depth–distance recorder, a pressure sensor and a propeller-driven device, which records on a smoked glass slide the distance travelled vs. depth (see Pearcy and Laurs, 1966).

Oblique tows of the trawl during nighttime hours were made to approximately 200 m depth at about monthly intervals from February 1964 to July 1967 at stations on a line running west from Newport, Oregon (44°39′N). Stations 15, 25, 45, and 65 nautical miles from shore were occupied regularly. Stations as far as 450 nautical miles from shore were sampled less frequently; one or more of these offshore stations was sampled in about one-third of the months. Normally one tow, sometimes two, but infrequently more, were made at a station. At stations 15 and 25 nautical miles from shore the trawl was towed to depths less than 200 m because of the shallowness of the water at these stations.

Specimens were fixed in 10% formalin in sea water at sea; later they were transferred to 36% isopropyl alcohol in the laboratory.

Determination of Reproduction State

Gonads were dissected from fish collected between July 1964 and November 1965. All available fish longer than 60 mm were examined. Fish longer than about 40 mm had gonads that could easily be categorized as either ovary or testis. Testes were lobate, white in color, and seminal vesicles were seen in more mature males at the posterior confluence of the seminal ducts. Ovaries were skeins of opaque yellow eggs, ranging from 0.05 to 0.70 mm in diameter.

Mean egg diameter was used as an index to reproductive state. At least 10 eggs were measured per ovary. The size distribution of developing eggs was similar in various parts of the ovary. When large developing eggs were in a matrix of small (less than 0.10 mm) eggs, only large eggs were measured. When two sizes of developing eggs (greater than 0.10 mm) were found in an ovary, 10 eggs of each size were measured to give two mean egg diameters for the fish.

Age Determination

Length-frequency analysis

Fish were measured (standard length) to the nearest millimeter, grouped into 5-mm length categories, and the numbers of fish in each length category from all the tows during a given month

were added together. Only fish greater than 20 mm in length were recorded in this part of the study because (1) the 20- to 25-mm length category is represented by the greatest numbers found for any length category, hence is probably the smallest size that is sampled adequately; and (2) according to Fast (1960), Pertseva-Ostroumova (1964), Ahlstrom (1963), and our own observations, *S. leucopsarus* larvae metamorphose at about 18 mm length. Thus 20- to 25-mm fish are the youngest juvenile fish.

The probability-paper method of graphical analysis of polymodal distributions, first suggested for aging members of a population by Harding (1949), was used to analyze length-frequency distributions. This method not only helps to separate overlapping size groups but also gives an estimate of the mean, standard deviation, and standard error of the mean for each size group.

OTOLITH ANALYSIS

Saccular otoliths were dissected from frozen fish, dried, immersed in xylene, and examined under a dissecting microscope at 66 magnifications using both transmitted and reflected light. Alternate use of both kinds of light aided the deciphering of the layers in larger otoliths. Annulus diameters were measured with an ocular micrometer along the longest dimension of the otolith.

RESULTS

REPRODUCTION

The season of spawning and the size (hence age) at which *S. leucopsarus* matures were estimated primarily from egg diameters and changes in reproductive state and secondarily from pulses in the recruitment of small fish. Stages of gonad maturity followed Nikolsky's (1963) "universal scale":

Stage I	No eggs visible; not distinguishable as an ovary.	
Stage II	Less than 0.10 mm; eggs are yellow, opaque.	
Stage III*a*	0.10–0.20 mm	Eggs are yellow and opaque in stage III.
III*b*	0.20–0.40 mm	
III*c*	0.40–0.60 mm	
Stage IV	Greater than 0.60 mm; eggs are translucent, granular, and grey, containing globular yellow bodies.	

The maturity stages of fish in different length categories during various months of the year are entered in Table 1. When ovaries had two sizes of developing eggs, the stage of maturity indicated by the larger eggs was noted. During 1964–65 females greater than 40 mm in length were all in stage II until October when fish longer than 65 mm began to reach stage III. Stage III fish occurred from October to April. Only two ripe fish (stage IV) were observed, one each in December and March. By April 1965 only stage II and early stage III fish were found. From May to September 1965 only stage II fish were noted.

These results suggest that *S. leucopsarus* becomes reproductively mature when it reaches about 65 mm length (later results indicate that this length corresponds to 4 years of age), maturation of gonads begins in October, and spawning occurs between December and March.

The occurrence of ovaries with two sizes of developing eggs was high in December, January, and February, and intermediate in March (Table 2). Such ovaries were not found between March and December. Since no large eggs were observed in fish collected during summer months, the two sizes of eggs in an ovary do not indicate 2 years' spawn. More than one spawning

TABLE 1. Maturation stages of eggs and ovaries of *Stenobrachius leucopsarus* during 1964–65. Arabic numerals in parentheses indicate the number of fish examined for each entry.

	1964				1965						
Length (*mm*)	July–Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May–Sept.	Oct.	Nov.
91–95	(1)II					(1)III*c*					
86–90									(2)II		
81–85	(3)II	(1)II (1)III*a*		(2)III*b* (4)III*c*	(1)III*c*	(4)III*b* (2)III*c*	(1)III*b*	(2)II	(5)II	(1)III*b*	
76–80		(1)II (1)III*a* (1)III*b*	(2)III*b*	(2)III*b* (1)III*c* (1)IV	(2)III*c*	(1)III*b* (1)III*c*	(1)III*b*	(2)II	(10)II		(1)III*b* (1)III*c*
71–75		(1)III*b*	(1)III*b*	(4)III*b*	(1)III*b*	(4)III*b* (1)III*c*	(2)III*b* (1)III*c* (1)IV	(1)III*a*	(11)II	(1)II (1)III*b*	
66–70	(1)II	(1)II	(2)II	(2)II (5)III*b*	(1)II (5)III*b*	(2)III*a* (1)III*b* (1)III*c*	(4)II (1)III*b*	(1)III*a* (3)II	(7)II	(1)II	(1)II (1)III*a*
61–65	(1)II	(3)II	(4)II	(6)II	(1)III*a*	(4)II	(1)II	(3)II	(3)II		(1)II
41–60	(2)II	(9)II	(5)II	(21)II	(9)II	(3)II	(12)II	(15)II	(15)II	(14)II (3)I	(2)II
20–40									(10)I		

TABLE 2. Percent of females greater than 65 mm in length with two sizes of eggs in ovaries.

Month	No. females observed	No. ripening females observed	% with two sizes of eggs in ovaries
	1964		
July	5	0	0
Aug.	1	0	0
Oct.	6	4	0
Nov.	5	3	0
Dec.	21	19	28
	1965		
Jan.	10	10	40
Feb.	19	18	42
Mar.	14	9	14
Apr.	10	2	0
June	13	0	0
July	19	0	0
Aug.	3	0	0

during the same season is also unlikely because the decline in occurrence of ovaries with two sizes of eggs coincided with the general decline in occurrence of ripe females in March and April. The smaller eggs are probably either ex-

pelled as immature gametes during spawning or are resorbed (see Nikolsky, 1963).

Recruitment of young fish 20–25 mm in length into trawl samples is also seasonal (Fig. 1). This is additional evidence for seasonal spawning: if spawning

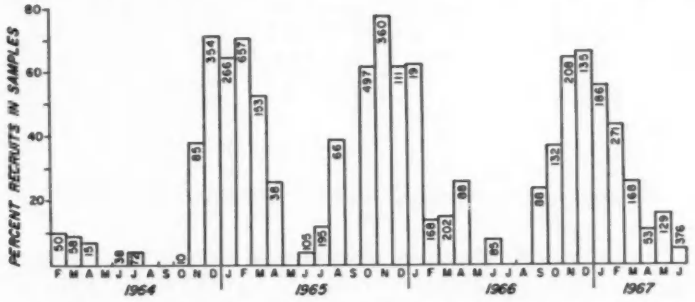

FIG. 1. Percent recruits (20- to 25-mm fish) in monthly midwater trawl samples. Numbers inside bars are the total number of *Stenobrachius leucopsarus* in the sample.

were spread over all seasons it would be expected that recruitment of young fish would continue throughout the year.

GROWTH

The average growth of *S. leucopsarus* was described by three methods: (1) the increase in mean length of year-classes from successive samples; (2) the mean lengths of fish aged by otolith analysis; and (3) the lengths of fish in different age-groups calculated back from the diameters of otolith annuli. In all methods a regression curve was fitted to the mean lengths.

LENGTH-FREQUENCY ANALYSIS

Length-frequency analysis is based on the tendency for the lengths of fish of one age to form a normal distribution; thus in a sample of a population each peak or length class corresponds to an age-group. This method assumes that: (1) spawning of year-classes is isolated enough in time so that the length frequencies of succeeding year-classes do not overlap too much; (2) members of a year-class all experience roughly the same growth conditions so that the length-frequency distribution of a year-class is not polymodal; and (3) all year-classes are represented in the samples well enough that incorrect ages are not assigned to length classes due to the absence of one or more year-classes in the sample (Rounsefell and Everhart, 1953).

Graphical analyses of monthly samples gave estimates of length-class means and their standard errors (Fig. 2). Recruitment of juveniles occurs in the early months of each year, and each year-class is discernible until sometime between its third and fourth birthday. The mean lengths less than 70 mm for each year-class were superimposed on a common time scale using January of

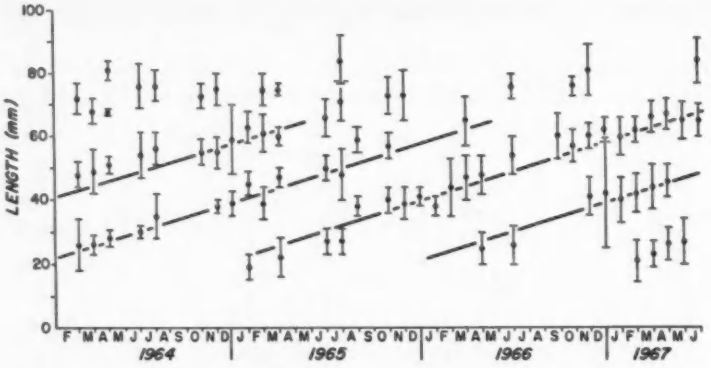

FIG. 2. Length group means from midwater trawl samples. Vertical lines indicate ±1 SD. Oblique lines are length = 20.78 × 1.59 (months) for fish under 70 mm.

the year following spawning for each year-class as the initial point. Linear regression of length with time gave:

$$\text{length} = 20.78 + 1.59X$$
$$r = 0.98$$

where r is Pearson's correlation coefficient and X is months numbered beginning with January of the year following spawning and length is in millimeters. This line is drawn in Fig. 2 as a reference line by repeating it with the same slope and "January intercept" for each of the year-classes represented. Attempts to fit a curvilinear relation to the mean lengths of age-groups I–III using computer techniques were unsuccessful because the means too closely approximated a straight line.

Comparison of the year-classes to this reference line shows that growth of *S. leucopsarus* is approximately linear during the 2nd, 3rd, and 4th years. Growth rates are similar. Each year-class passes through the same length categories during the same months of the year.

OTOLITH ANALYSIS

Otoliths consist of alternately opaque and translucent concentric layers. Age can be determined if an annular pattern of alternation of these layers exists, the number of alternations giving the fish's age. Such a pattern is demonstrated for *S. leucopsarus* in Table 3: the largest percentages of otoliths with opaque peripheries occurred during summer months, the largest percentages with translucent peripheries during winter. The reason that no month showed 100% opaque peripheries is that the otolith margin may appear to be translucent because of its thinness even though an opaque layer is forming.

Translucent layers are forming on otoliths during January, approximately the time of spawning of *S. leucopsarus* (Table 3). The outermost edge of opaque

TABLE 3. Percentage of otoliths with translucent or opaque peripheries or both during various months of the year.

Date	Translucent (%)	Opaque and translucent (%)	Opaque (%)	No. examined
Jan. 1966	100	0	0	2
Feb. 1966, 1969	70	18	13	55
Apr. 1966	62	13	25	8
June 1966, 1969	27	32	41	22
Oct. 1965, 1968	43	31	25	28
Nov. 1968	95	0	5	38
Dec. 1966	71	0	29	21

layers, however, is the most conveniently measured annual demarcation in otolith structure. These are called "annuli" even though their formation does not coincide with the birthdays of the fish. Opaque layers are formed in summer about 6 months before the corresponding birthday.

The opaque core of the otolith was considered to be deposited during the 1st year of life. Fish belonging to the recruit length category (20–25 mm) have an otolith consisting of an opaque center surrounded by some translucent material. Based on our length-frequency data (Fig. 1, 2) and those of Fast (1960), these individuals are about 1 year old.

Although otolith analyses indicated some fish may be 7 or 8 years old, otoliths from fish older than 5 years could not be analyzed with reliability owing to the merging of peripheral annuli. Photographs of otoliths from age-groups I–IV with both translucent and opaque peripheries are shown in Fig. 3.

The mean lengths of age-groups of fish aged by otoliths were fitted to the von Bertalanffy equation (von Bertalanffy, 1938) (Fig. 4A) giving these estimates:

$$L_\infty = 84.96, \quad SE = 2.79$$

$$L_\infty - L_0 = 79.32, \quad SE = 3.12$$

$$k = 0.34, \quad SE = 0.044.$$

Thus

$$L_X = 84.96 - 79.32e^{-0.34X}$$

$$\text{Variance} = 3.06$$

where L_X = the length at age X; L_∞ = the asymptotic length; L_0 = the length at age zero; e = the base of the natural logarithms; k = the rate at which length approaches the asymptote; and X = age in years.

Age-groups V–VII were included. Though confidence in these age determinations is less than for the first four age-groups, the mean lengths of fish in

these age-groups are consistent with the trend established by the younger age-groups.

BACK CALCULATION OF AGE-GROUP LENGTHS

Diameters of otolith annuli were used to compute lengths of fish at earlier ages by deriving a formula relating the logarithm of annulus diameter to the logarithm of standard length (see Rounsefell and Everhart, 1953).

Mean lengths of age-groups I–IV calculated by this method were fitted to the von Bertalanffy equation (Fig. 4B) giving these estimates of the parameters:

$$L_\infty = 75.01, \quad \text{SE} = 5.00$$

$$L_\infty - L_0 = 84.17, \quad \text{SE} = 3.01$$

$$k = 0.377, \quad \text{SE} = 0.0629.$$

Thus

$$L_X = 75.01 - 84.17e^{-0.377X}$$

$$\text{Variance} = 1.468.$$

SYNOPSIS OF GROWTH DETERMINATIONS

The three growth curves, derived from length-frequency analysis, otolith analysis, and back calculations, are superimposed in Fig. 5. Agreement among the curves is good.

GROWTH IN WEIGHT

Fish from which otoliths were to be removed were dried to a constant weight on glass slides in an oven at 65 C (3 or 4 days), scraped from the slide, and weighed to the nearest milligram. Linear regression of the logarithm of weight (in grams) on the logarithm of length (in centimeters) gave:

$$\log \text{wt} = -2.898 + 3.595 \log \text{length}$$

or

$$\text{wt} = 0.001264\ (\text{length})^{3.595}.$$

The growth curve obtained from otolith analyses is transformed into growth in weight in Fig. 6 by this relation.

DISCUSSION

Fast (1960), in his comprehensive study in Monterey Bay, California, found growth and reproduction patterns similar to those off Oregon (Fig. 7). He found ripening females during the entire year but concluded that spawning is seasonal in intensity from the seasonal variation in abundance of young size groups. Spawning occurred from November through August, compared with October through March off Oregon.

Fast estimated that age-group I fish (in their 2nd year) average 32 mm standard length; age-group II, 50 mm; and age-group III, 66 mm. These esti-

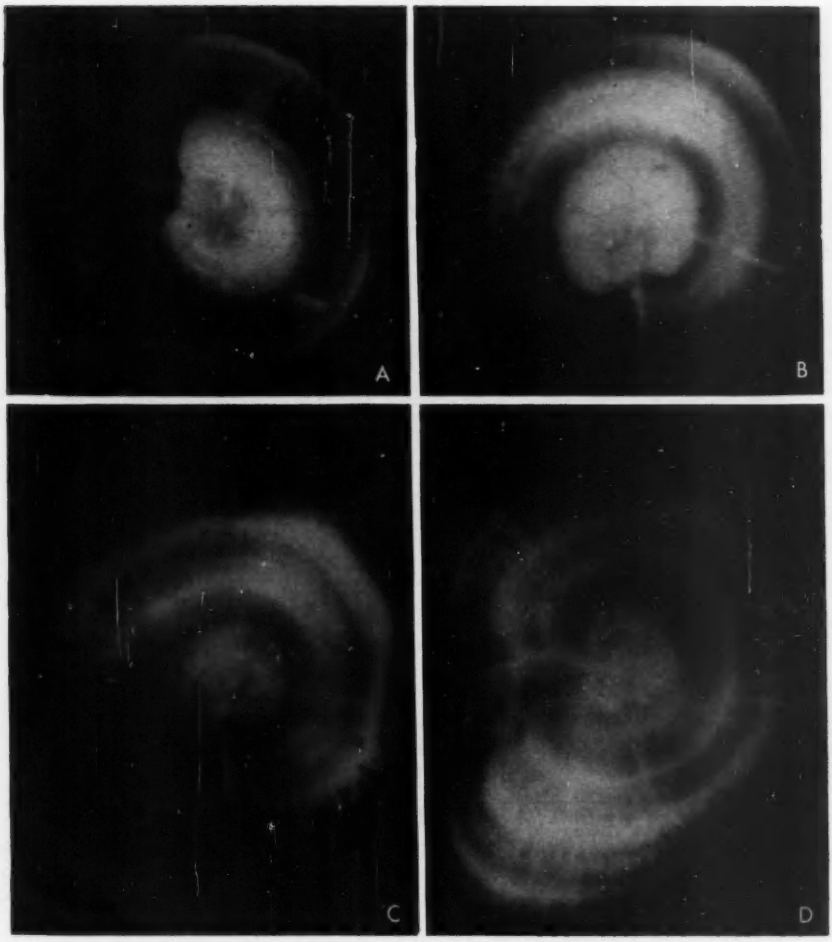

FIG. 3. *Stenobrachius leucopsarus* otoliths.

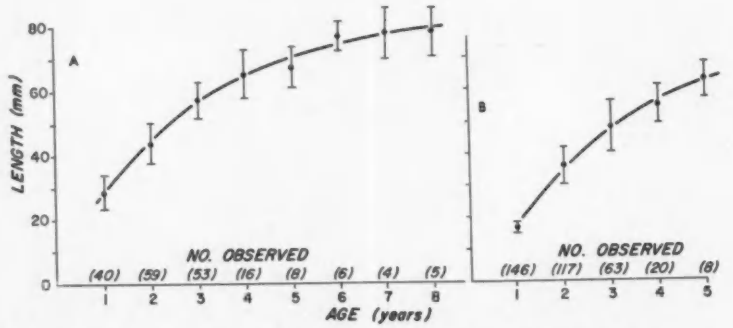

FIG. 4. (A) Means and standard deviations of lengths in otolith analysis age-groups. (B) Means and standard deviations of lengths of age-groups back-calculated from otoliths. Curves fitted using computer program CURVFIT at Oregon State University.

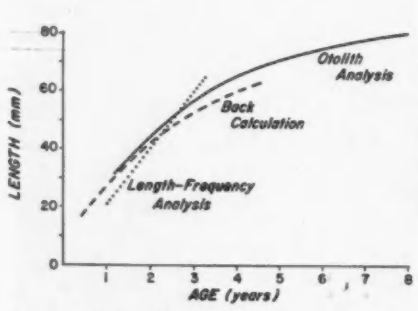

FIG. 5. Comparison of three growth curves, Fig. 2, 4A, B.

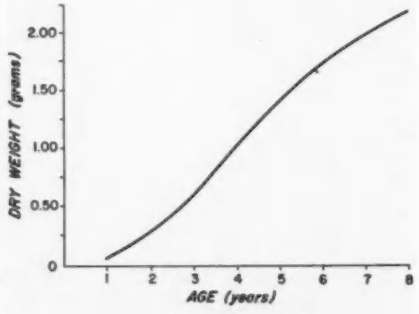

FIG. 6. Growth in weight.

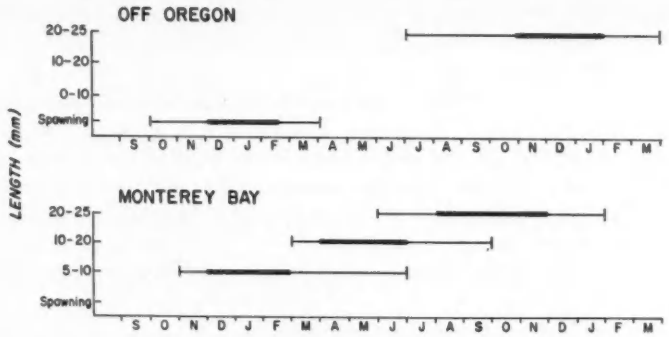
OFF OREGON
20-25
10-20
0-10
Spawning
S O N D J F M A M J J A S O N D J F M
LENGTH (mm)
MONTEREY BAY
20-25
10-20
5-10
Spawning
S O N D J F M A M J J A S O N D J F M

FIG. 7. Comparison of periods of spawning, catches of larvae, and catches of small juveniles in Monterey Bay and off Oregon. Thin lines indicate ranges of occurrence, thick lines periods of abundance.

mates agree precisely with the regression line fitted to length-frequency data from Oregon collections (Fig. 8). Although Fast did not assign ages to large fish, examination of his data suggests that age-group IV fish are about 72 mm and age-group V are about 76 mm. These estimates agree fairly well with our growth curve based on otolith analyses.

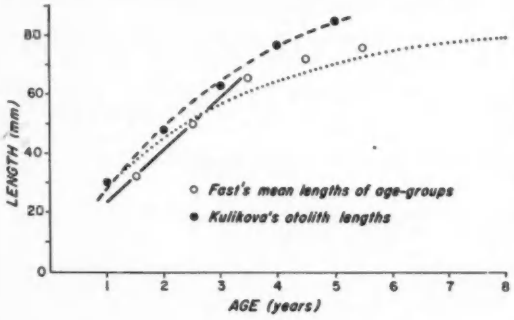

FIG. 8. Comparison of growth curves from Kulikova's otolith analysis, Fast's length-frequency analysis, our length-frequency analysis, and our otolith analysis. Broken line: fit of the von Bertalanffy equation to Kulikova's data, length $= 111 - 111\ e^{-0.29(age)}$; dotted line: fit of the von Bertalanffy equation to our otolith analysis, length $= 85 - 79\ e^{-0.34(age)}$; solid line: our length-frequency regression line, length $= 21 + 19$ (age $- 1$).

Kulikova (1957) examined otoliths from 46 *S. leucopsarus* collected in the western North Pacific. For comparison, the von Bertalanffy equation was fitted to the mean lengths of age-groups in Kulikova's study (Fig. 8). The estimate of L_∞ (111 mm) is much higher than in this study, the estimate of k (0.29) somewhat lower. Kulikova's age estimates are similar to ours and those of Fast for the first 3 years of growth but they indicate faster growth for age-groups IV and V (Fig. 8). Her estimates and Bolin's (1956) are similar for age-groups I–IV.

This discrepancy in size and growth of older fish as well as Fast's failure to age large fish is significant in pointing out the difficulty in assigning ages to large fish. Neither method, length-frequency analysis, or otolith analysis provides an entirely confident determination.

Odate (1966) and Halliday (1970) estimated the age and growth of the lanternfishes *Myctophum affine* (Lütken) and *Benthosema glaciale* (Reinhardt) respectively. *Myctophum affine* grows rapidly during the first year attaining a length of 36 mm; then growth slows down. It spawns at 3 years of age and reaches a maximum length of 78 mm. The growth rates of *B. glaciale* and *S. leucopsarus* are similar ($k = 0.36$ vs. 0.34), but *B. glaciale* spawns at only 2 years of age, and it attains a smaller maximum length (68 mm) and a lower maximum age ($4\frac{1}{2}$ years) than *S. leucopsarus* off Oregon. Halliday reached opposite conclusions, however, when comparing *B. glaciale* and *S. leucopsarus* because he only based his comparisons on Bolin's (1956) preliminary report.

ACKNOWLEDGMENTS

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Effects of Copper on *Gammarus pseudolimnaeus*, *Physa integra*, and *Campeloma decisum* in Soft Water

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Three invertebrate species were subjected to acute (96-hr), followed by long-term (6-week) copper exposures under continuous-flow bioassay conditions. Survival, growth, reproduction, and feeding were the responses used for measuring toxicant effects. The average 96-hr median tolerance limits (TL_m) values for *Campeloma decisum*, *Physa integra*, and *Gammarus pseudolimnaeus* were 1.7, 0.039, and 0.020 mg/liter total copper, respectively. The total copper concentration having no effect after 6 weeks exposure for all three species was between 8.0 and 14.8 μg/liter. The newly hatched amphipods obtained from the second 6-week study were exposed to copper for 9 additional weeks, and grew to the adult stage only in copper concentrations ≤4.6 μg/liter. After 5 weeks exposure to a water system, survival of newly hatched *Gammarus* was markedly reduced when copper concentrations were between 12.9 and 6.2 μg/liter.

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INTRODUCTION

NUMEROUS STUDIES have shown that the addition of copper salts to natural waters can seriously threaten aquatic life. Copper sulfate is widely used in industry and for controlling unwanted vegetation. Much of the previous work on copper toxicity, summarized by McKee and Wolf (1963), is based on short-term exposures to determine lethal and other end points, with results ranging from 0.015 to 3.0 mg/liter for fish and aquatic invertebrates. Water characteristics such as *p*H and hardness have been shown to alter the levels of most heavy metals needed to kill or impair the continued survival of aquatic animals (Skidmore, 1964). In addition, little is known about the effect of natural and unnatural substances (chelating agents and others) on copper toxicity. Until chemical methods are developed for measuring very small amounts of ionic and combined forms of copper present in solution, contributions in this toxicity field will be largely limited to defining the amounts of total copper affecting aquatic life in a particular body of water.

In a previous study (Arthur, 1970), three invertebrate species were submitted to acute (96-hr) followed by chronic (6 weeks and longer) exposures of linear alkylate sulfonate detergent. The purpose of the present study was to define the effects of copper under similar test conditions on feeding, growth, reproduction, and survival of the same three invertebrate species.

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EXPERIMENTAL METHODS

All tests, except for a series of growth experiments with newly hatched amphipods, were carried out under continuous-flow conditions with a serial diluter (Mount and Warner, 1965) constructed and calibrated to give a dilution factor of 0.5. Water delivery systems consisted of glass, PVC plastic, silicone rubber adhesive sealant, black laboratory rubber, and stainless steel materials. Test tanks were 4-gal glass aquaria, with 7 inches (17.5 cm) of water depth, and holding approximately 12.5 liters. A glass baffle fitted to the end opposite the tank's standpipe permitted water to be delivered to the bottom fifth of the tank.

Duplicate test tanks were used for all copper concentrations. Rate of flow to each was 30–40 ml/min, except the lowest test concentration, for which rate of flow was about 75 ml/min. Durotest (Optima FS)[1] and wide spectrum Grow-lux fluorescent tubes provided light of approximately 60 ft-c of illumination at the water's surface. Lights were automatically controlled, and the daily photoperiod was set for seasonal light conditions at Duluth, Minnesota. Water for the 6-week adult trials was obtained from Lake Superior through the city of Duluth, Minnesota, pumping station. In the laboratory the water was carbon-filtered to remove chlorine (as chloramines). Sodium thiosulfate was added continuously to remove residual chloramines; the level of sodium thiosulfate in the finished water was approximately 100–200 μg/liter. Water for the remaining studies was piped directly into the laboratory from Lake Superior.

The test species were the amphipod *Gammarus pseudolimnaeus* Bousfield and the snails *Campeloma decisum* (Say) and *Physa integra* (Haldeman). All animals were collected from the St. Croix and Eau Claire rivers in the vicinity of Gordon, Wisconsin. Acclimation time in the laboratory in holding tanks at 15 C varied from 10 days to 5 weeks.

Ten animals of each species were used in each tank, so that a total of 20 individuals of each species was tested at each concentration. Complete immobilization was considered the end point for the amphipods and failure to respond to probing was the end point for the snails. Before the chronic tests were started, standard procedures (American Public Health Association, 1960) were followed for determining the 96-hr median tolerance limits (TL_m). Each 96-hr TL_m value was determined twice. Total copper concentrations were measured at the beginning and end of each actual test. Two 6-week studies were performed for each of the three species at a test temperature of 15 ± 1 C. Animals were fed during the long-term experiments only. The operculate snails were fed freshly killed unionid clams, the pulmonate snails lettuce and cerophyll, and the amphipods maple and aspen leaves presoaked in diluent water. Shell lengths of operculate and pulmonate snails were measured with calipers (0.1-mm accuracy) and a ruler (0.5-mm accuracy), respectively, at the beginning and end of the test. *Physa* and *Campeloma* snails ranged initially in size from 4 to 7 mm and 11 to 27 mm, respectively. Only adult *Gammarus* were used in each 6-week test. After termination of the second 6-week trial, the newly hatched *Gammarus* were counted and 10 reared to adults in each respective test chamber at 18 ± 1 C.

Ten 1- to 3-day-old newly hatched *Gammarus pseudolimnaeus* were reared in 400-ml beakers containing 250 ml of test water from each of the diluter's 12 chambers. These young amphipods were obtained from adults kept in the 15 C holding tanks. The test tanks served as a constant temperature water bath for the beakers. Two-thirds of the beaker water was changed twice each week and the water completely changed once each week, at which time a mortality count was taken. An aspen leaf was left in each beaker for the experiment's duration. Ten milliliters of a cladoceran and green algal culture were added once each week when the test water was completely changed.

The stock copper solutions were prepared by dissolving 0.11 g of anhydrous $CuSO_4$, ACS grade, in 10 liters of distilled water and acidified with 3–4 drops of concentrated sulfuric acid prior to diluter use. This same stock concentration was prepared for all the long-term studies. From one duplicate tank for each concentration, a 100-ml sample of water was collected daily 5 days a week and acidified upon initial collection with redistilled 1:1 HCl. Fifty milliliters of water were taken from the beakers 4 days a week. Water analyses of the combined samples were alternated weekly between duplicate concentration tanks.

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[1]Mention of commercial products does not constitute endorsement.

Copper concentrations in the combined samples were determined by atomic absoprtion spectrometry after preconcentration by solvent extraction. Copper was chelated by adding 10 ml of a 1% aqueous solution of the ammonium salt of nitrosylphenylhydroxylamine (cupferron) to a 200-ml water sample (Takeuchi et al., 1966). After adjusting *p*H, if necessary, to the range 3.0–7.5, 30 min were allowed for the chelate to form and 10.0 ml of methyl isobutyl ketone (MIBK) added. The samples were shaken for 10 min on a mechanical shaker and layers separated. A second extraction was performed with MIBK. The combined MIBK extracts were aspirated directly into the burner on the atomic absorption instrument. The samples were aspirated into a laminar flow (10 cm) Perkin-Elmer burner mounted on a Beckman Model 979 atomic absorption spectrophotometer. Instrument settings, with the exception of fuel flow, were those recommended by the manufacturer. With the solvent aspiration rate maximized (4–5 ml/min), fuel flows were adjusted as rich as possible without producing a luminous flame. Glass-distilled water or tap water with added copper was extracted under the same conditions as the water samples to prepare standard curves. Substracting absorbance contribution by copper contained in tap water produced similar standard curves for the two waters. Minimum detectable copper concentrations by this procedure was approximately 0.5 μg/liter. Since all water samples were acidified prior to analysis, copper concentrations reported in this paper represent total copper values.

Routine determination of water chemistry was generally conducted once a week during the long-term studies on one or two tanks (Table 1). Dissolved oxygen, *p*H, total hardness, acidity, alkalinity, and chloramines were measured by standard procedures (American Public Health Association, 1960). The measured level of total chloramines in the city water after carbon filtration (in line, prior to diluter use) averaged 0.004 mg/liter and ranged from 0.0 to 0.019 mg/liter (N = 55). Actual chloramine concentrations in the test tanks were probably lower because of greater contact time. Measurements of the weekly total copper concentrations in combined samples from the test tanks and beakers are presented in Table 2. Recovery of known amounts of added copper in glass-distilled water were routinely performed each week and averaged 95–100% throughout the study. Similar recovery results were obtained from copper added to the diluent water.

RESULTS

Acute Toxicity Tests

The average 96-hr TL_m values were 1.7 (2.0 and 1.4), 0.039 (0.041 and 0.037), and 0.020 (0.022 and 0.019) mg/liter for *Campeloma decisum*, *Physa integra*, and *Gammarus pseudolimnaeus*, respectively. In general, the operculum of *Campeloma* was tightly closed within the shell aperture in total copper concentrations greater than 1.0 mg/liter, but motility about the tank after 24 hr was observed only with concentrations less than 0.03 mg/liter. *Physa integra* was not motile in tanks of total copper concentrations greater than 0.03 mg/liter. The amphipods, when placed in total copper concentrations of more than 0.02 mg/liter, immediately exhibited increased locomotor activity but lost their powers of motility prior to death.

Long-Term Toxicity Tests

Survival of adults of all three test species during the 6-week exposure tests was markedly reduced in the two highest copper concentrations, 14.8 and 28.0 μg/liter (Table 3). Growth of *Physa* and *Gammarus* (as determined from shed exuviae) was approximately the same in copper concentrations of 8.0 μg/liter or less, but no growth occurred for either species in the 14.8 and 28.0 μg/liter concentrations. Although growth was not measurable for

TABLE 1. Chemical characteristics of test tank water.

Pairs of test chambers	pH			Dissolved O_2 (mg/liter)			Total hardness (as $CaCO_3$) (mg/liter)			Acidity (mg/liter)			Total alkalinity (as $CaCO_3$) (mg/liter)		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Ra
1	38	7.66	7.10–8.06	27	9.6	7.9–10.6	23	44.6	38–48	6	1.0	0.7–1.5	7	43.0	40
2	38	7.70	7.53–8.06	24	9.7	8.5–11.1	20	44.2	35–50	4	1.0	0.8–1.5	8	43.0	40
3	33	7.70	7.41–8.15	22	9.6	8.2–11.0	17	45.6	43–52	5	0.9	0.5–1.5	6	42.7	40
4	29	7.68	7.35–7.96	23	9.6	8.5–10.8	19	44.9	41–50	5	1.0	0.8–1.6	9	42.9	40
5	30	7.70	7.40–8.14	23	9.5	8.2–10.8	19	45.1	41–55	4	0.9	0.8–1.1	8	42.5	40
6 (Control)	24	7.73	7.42–7.98	26	9.5	8.6–11.0	21	45.1	40–50	5	1.0	0.5–2.0	10	42.2	40

TABLE 2. Measurement of total copper concentrations in test tanks and beakers as measured from weekly combined samples (μg/liter).

Pairs of test chambers	N	Mean±SD	Range
1	32	28.0±4.9	43.2–17.8
2	34	14.8±4.0	27.5–9.9
3	34	8.0±2.7	16.9–4.0
4	34	4.6±1.9	9.7–2.2
5	31	2.9±1.4	7.5–1.2
6 (Control)	26	2.0±1.2	5.7–0.7
Pairs of test beakers			
1	9	23.9±3.6	31.6–17.0
2	9	12.9±2.1	16.0–9.0
3	9	6.2±1.4	8.0–3.2
4	9	3.7±1.2	4.8–0.8
5	9	2.8±0.4	3.5–2.0
6 (Control)	9	1.9±0.8	3.0–0.3

the *Campeloma* snails, these animals readily accepted and completely devoured fresh unionid clam meat in concentrations up to 8.0 μg/liter, but would not accept or consume this food in the 14.8 and 28.0 μg/liter test tanks. Another generation of *Physa* was produced in copper concentrations of 8.0 μg/liter and less. The snail results indicate a 6-week no-effect level of between 8.0 and 14.8 μg/liter for adults.

A second generation of *Gammarus* was produced in copper concentrations of 8.0 μg/liter and less during both of the 6-week adult trials (Table 4). At the termination of trial 2, the numbers of F_1 *Gammarus* produced were counted and 10 of the newly hatched gammarids were allowed to remain in each tank. After 9 weeks additional exposure the newly hatched amphipods reached adult size only in copper concentrations of 4.6 μg/liter and less (60% survival

TABLE 3. Percentage survival of test species after 6 weeks exposure to total copper.

Mean total copper concn (μg/liter)	Duplicate tanks	Test 1			Test 2		
		Gammarus pseudolimnaeus	*Campeloma decisum*	*Physa integra*	*Gammarus pseudolimnaeus*	*Campeloma decisum*	*Physa integra*
28.0	A	0	0	0	0	10	0
	B	0	10	20	0	10	0
14.8	A	0	40	30	10	30	30
	B	10	50	40	20	40	40
8.0	A	40	90	60	50	100	40
	B	50	70	50	60	90	60
4.6	A	50	90	50	50	80	70
	B	30	100	40	70	100	60
2.9	A	60	100	40	60	70	50
	B	30	100	50	70	90	50
2.0 (Control)	A	90	90	60	50	80	30
	B	50	100	60	60	90	60

TABLE 4. Numbers of newly hatched *Gammarus pseudolimnaeus* produced from trial 2 and numbers surviving to adult stage after 9 weeks additional exposure.

Mean total copper concn (μg/liter)	Duplicate tanks	No. young produced	No. continued in treatment	No. surviving to adults
28.0	A	0	–	–
	B	0	–	–
14.8	A	2	2	0
	B	0	–	–
8.0	A	29	10	0
	B	25	10	0
4.6	A	50	10	7
	B	49	10	5
2.9	A	109	10	7
	B	117	10	6
2.0 (Control)	A	57	10	8
	B	44	10	8

at 4.6 μg/liter, 65% at 2.9 μg/liter, and 80% at 2.0 μg/liter). Thus this species was tested over a one-generation cycle (adult to adult) with all life stages exposed to copper. Under the test conditions used in this study the safe total copper concentration for *G. pseudolimnaeus* was between 4.6 and 8.0 μg/liter with Lake Superior water.

In a static water system, survival of the newly hatched *Gammarus* was significantly reduced after 5 weeks exposure to copper concentrations of 23.9 and 12.9 μg/liter (Table 5). However, those amphipods surviving 5 weeks

TABLE 5. Percentage survival of newly hatched *Gammarus pseudolimnaeus* after 5 weeks exposure to total copper in static system.

Mean total copper concn (*μg/liter*)	Duplicate beakers	% survival		Avg no. molts (*growth*)
		Trial 1	Trial 2	
23.9	A	0	0	–
	B	0	0	
12.9	A	20	20	3.6
	B	20	10	
6.2	A	60	40	3.8
	B	90	70	
3.7	A	90	100	3.5
	B	90	80	
2.8	A	70	100	4.1
	B	70	60	
1.9 (Control)	A	40	80	3.9
	B	80	60	

exposure in 12.9 μg/liter grew at about the same rate as those in the lower copper concentrations. Growth was determined by Clemens' (1950) method of counting first and second antennal segments. The static tests establish a safe copper concentration for survival between 12.9 and 6.2 μg/liter, supporting results obtained under continuous-flow trials.

DISCUSSION

Previous toxicity studies for morphologically related species indicate that lethal and immobilization concentrations ranged from 0.01 to 1.25 mg/liter (Clemens, 1950; McKee and Wolf, 1963; Howard et al., 1964). However, none of these experiments extended over prolonged periods of time. Because of the test systems, diluent water characteristics, and a more sensitive analytical method employed in this study, the present data cannot be directly compared with known invertebrate toxicity values.

Most of the available information on the long-term effects of copper under continuous flow conditions pertains to fish. Mount (1968), using diluent water that had a total hardness of 198 mg/liter (as $CaCO_3$), found that the maximum acceptable tolerance concentration (MATC) for the fathead minnow (*Pimephales promelas*) was between 15 and 33 μg/liter copper. In a later study with fathead minnows in soft water (total hardness of 31 mg/liter, $CaCO_3$), Mount and Stephan (1969) observed that the MATC was between 10.6 and 18.4 μg/liter. Thus, the safe level of copper for fathead minnows under similar testing conditions as used for this study was approximately twofold greater than for *Gammarus pseudolimnaeus*. In a study with Atlantic salmon (*Salmo salar*) parr in water having a total hardness of 14.8 mg/liter (as $CaCO_3$) and using a preconcentration extraction method, Sprague (1964) showed that in the laboratory these fish avoided copper concentrations of 4–5 μg/liter. The

lethal threshold for salmon in this water was estimated to be 44 μg/liter copper. Hubschman (1967) studied crayfish (*Orconectes rusticus*) survival to copper under continuous-flow conditions and observed that age markedly influenced the acute toxicity level. Newly hatched young were about 15 times more sensitive to copper than adults. A calculated concentration of 15 μg/liter affected growth of these young after 30 days. Summarily, these results indicate that all the test species were extremely sensitive to copper, and safe levels approached those already existing naturally in soft water.

Despite the noticeably different 96-hr TL_m values between *Physa* and *Campeloma*, the 6-week no-effect concentrations were the same. This difference in acute sensitivity is attributed to the ability of *Campeloma* to use the operculum as a defensive reaction in "sealing" itself from the copper environment. Probably most operculate snails would be able to withstand high short-term doses of copper that would eliminate *Physa* and perhaps many other pulmonate snails.

Soft Lake Superior water, used as the diluent source in this study, has relatively low natural amounts of dissolved materials and ionic compounds that might alter copper toxicity. The results of this study can serve as a baseline for the maximum toxicity of total copper under the conditions described.

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Morphology of Certain Viruses of Salmonid Fishes. I. In vitro Studies of some Viruses Causing Hematopoietic Necrosis

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An electron microscope study was performed on three virus isolates that caused hematopoietic necrosis in salmonid fishes: infectious hematopoietic necrosis (IHN), Oregon Sockeye Disease (OSD), and Sacramento River Chinook Salmon Disease (SRCD). All three isolates were examined by negative staining of fathead minnow (FHM) monolayer tissue culture concentrates and IHN virus was also examined in thin sections of FHM cells. Virus-like particles were observed in infected tissues, but similar structures were not found in uninfected cultures. All three isolates were bullet-shaped, but oval and truncated forms were also observed. Mean measurements of particles from IHN-virus-infected tissue were 158 × 90 mμ. They consisted of an outer coat 15 mμ thick, a core 60 mμ in diameter, subunits about 5 mμ, and an axial pore about 20 mμ in diameter. These particles also were seen budding from the cytoplasmic membrane. Similar particles from SRCD were 159 × 90 mμ and isolates from OSD were 181 × 91 mμ. The three isolates were morphologically indistinguishable from one another and the greater length of OSD was considered insignificant. IHN, SRCD, and OSD viruses were tentatively placed in the rhabdovirus group, but serological studies are needed to determine if they are antigenically identical or should be included as separate members. Biochemical and physical characteristics of these viruses and a comparison with other salmonid viruses is also discussed.

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INTRODUCTION

AMEND ET AL. (1969) reported the decimation of a hatchery stock of juvenile sockeye salmon (*Oncorhynchus nerka*) and rainbow trout (*Salmo gairdneri*) by a viral disease. The virus was isolated in fathead minnow (FHM) monolayer cell culture and River's postulates were fulfilled. The first histopathological change in infected fish was necrosis of the hematopoietic tissues of the spleen and anterior kidney. As the disease progressed other tissues became involved.

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Histopathologic studies of the tissues of moribund fish showed necrosis of the pancreatic acinar and islet cells, granular cells in the stratum compactum of the alimentary tract, and focal necrotic areas of the liver. The disease was named "infectious hematopoietic necrosis" (IHN) because of the primary pathological changes.

Before the isolation of IHN virus, the only viruses known to cause epizootics in hatchery populations of salmonids were the viruses of viral hemorrhagic septicemia (VHS), Sacramento River Chinook Salmon Disease (SRCD), Oregon Sockeye Disease (OSD), and infectious pancreatic necrosis (IPN). The primary pathologic change in fish infected with IPN virus occurs in the pancreas whereas the primary lesion in the other salmonid virus diseases occurs in the hematopoietic tissues (Yasutake et al., 1965; Yasutake, 1968). Prior to this report only IPN and VHS had been studied with the electron microscope. In the present paper, the morphological characteristics of images that appear to be of IHN, OSD, and SRCD viruses are reported.

MATERIALS AND METHODS

Viruses

IHN virus was isolated from moribund sockeye salmon from Cultus Lake, B.C., in June 1967. Lyophilized OSD virus was obtained from Dr J. L. Fryer, Department of Microbiology, Oregon State University, Corvallis, Oreg., who isolated it originally from moribund sockeye salmon fingerlings in 1958. SRCD virus was isolated originally by Parisot (1965). The SRCD virus in the present study was isolated by Amend in 1967 from moribund chinook salmon (*Oncorhynchus tshawytscha*) fry that had been frozen at −80 C for 20 months. The three viruses were cultivated in cultures of fathead minnow (FHM) cells (Gravell and Malsberger, 1965) at 18 C.

FHM cells were grown in 50-cm² flasks in medium consisting of Eagle's Minimal Essential Medium (MEM) (Earle's balanced salt solution), 90%; agamma fetal calf serum, 10%; and 100 I.U. penicillin G and 100 μg streptomycin sulfate per milliliter. FHM cultures were inoculated with 2 ml of undiluted culture-grown virus. Virus was allowed to adsorb for 30 min at 18 C. The cell sheet was then washed twice with MEM containing 10% serum. The medium was replaced on the cells and the titer of cell-free virus was determined every 8 hr for 48 hr by tube dilution using four cultures for each step. Fifty percent end points of infectivity ($TCID_{50}$) were calculated by the method of Reed and Muench (1938). An alternate method for viral assay was plaque production in FHM monolayers because all three viruses form plaques in these cultures.

Preparation of Virus by Negative Staining

FHM cells were inoculated with IHN, OSD, or SRCD virus from the sixth to 10th culture passage. At appropriate intervals after inoculation, culture medium containing sloughed cells was harvested and centrifuged at 480 × g for 10 min at 4 C. In several experiments, the supernatant fluid was passed through a 0.45-μ filter that had been pretreated with 10% calf serum to prevent adsorption of the virus onto the membrane (Ver et al., 1968). One or two drops of 4% osmium tetroxide per 10 ml of filtrate were added to aid in the preservation of the virus particles that are apparently very fragile. The virus was then sedimented at approximately 100,000 × *g* for 1 hr at 4 C. The virus pellet was resuspended in a few drops of the supernatant fluid, mounted on a carbon-coated grid, treated with 2% sodium phosphotungstate and examined in an RCA EMU 3G electron microscope. Uninfected FHM cells treated similarly served as controls.

Preparation of Thin Sections of FHM Cells Infected with IHN Virus

FHM monolayers grown in 25 cm² Falcon tissue culture flasks were inoculated with 0.1 ml of undiluted MEM containing IHN virus. When cytopathological effects (CPE) were observed after 72 hr at 18 C, the medium was removed and a mixture of equal volumes of 4% osmium

tetroxide and medium 199 was added to the cells. The monolayers were fixed at room temperature for 40–60 min, dehydrated in graded alcohols, embedded in epoxy resin (Luft, 1961), sectioned, and stained with uranyl acetate either alone or followed by lead citrate.

RESULTS

Multiplication of IHN, OSD, and SRCD Viruses in FHM Monolayers

When IHN virus was inoculated onto FHM monolayers and incubated at 18 C, viral titers began to increase between 16 and 24 hr after inoculation and the first CPE was evident between 32 and 48 hr. Early CPE was characterized by increased basophilia of the nuclear membrane and decrease in the density of the nuclear chromatin. After 3 days, the nucleus became enlarged and often two or more nuclei were observed in one cell (Amend et al., 1969). At 4 days, the cells had become round and aggregated in grapelike clusters. The cells sloughed from the glass surface between 5 and 7 days. Maximum titers of about 1×10^6 $TCID_{50}$ per 0.2 ml occurred at this time. When cultures were incubated at a lower temperature (10 C) or if the virus inoculum was small, the events just described were delayed accordingly.

Wingfield et al. (1969) reported that OSD virus titer began to increase 24 hr after inoculation of this virus onto sockeye salmon embryonic cells. The initiation of SRCD virus multiplication has not been established. Our studies showed that OSD and SRCD viruses produced CPE in FHM cells that was indistinguishable from that produced by IHN virus. In addition, the maximum titer of approximately 10^6 $TCID_{50}$ per 0.2 ml was reached after a period of incubation similar to that described for IHN virus.

Morphology of IHN Virus

OBSERVATION OF NEGATIVELY STAINED PREPARATIONS

Negatively stained preparations of tissue culture material taken on the 3rd or 4th day after inoculation revealed very few viruslike particles. By the 7th day numerous particles suggestive of virus were present (Fig. 1). Most of the particles were bullet-shaped (Fig. 2 and 3B) but some were oval (Fig. 1, 2, and 3A) or short and hemispherical in shape (Fig. 2 and 3G). Similar particles were not observed in uninfected FHM cells.

Dimensions of the assumed virus particles are: mean diameter of 31 oval and bullet-shaped particles 90 mμ with a range of 80–100 mμ; mean length of the same particles 158 mμ with a range of 120–230 mμ. Mean diameter of 10 short particles was 94 mμ. The "length" from the flat surface to the opposite side gave a mean value of 92 mμ. Except for length, these particles were similar to the longer forms and were assumed to be truncated forms of the virus (T particles) similar to the truncated forms of vesicular stomatitis virus described by Huang et al. (1966).

Assumed virus particles consisted of an outer coat 15 mμ thick and a core approximately 60 mμ in diameter (Fig. 3C–E). Subunits measuring about

50 A were sometimes discernible in the outer coat (Fig. 3C, D, F). The arrangement of the subunits was indistinct. When either the bullet-shaped particles or the truncated forms were oriented with a face-on view of the flat end, an opening or pore about 20 mμ in diameter was seen (Fig. 2, 3F, G). Occasionally a structure extended outward from the area of the pore giving the appearance of a short tail (Fig. 3A, D).

OBSERVATION OF SECTIONED FHM TISSUE CULTURE CELLS INFECTED WITH IHN VIRUS

Sections of FHM cells inoculated with IHN virus 72 hr prior to fixation revealed very few viruslike particles even though many of the cells were degenerating. When FHM cells were fixed and embedded 7 days after inoculation with IHN virus, numerous bullet-shaped particles were observed (Fig. 4A–F). Particles were seen within the cytoplasm of degenerating cells (Fig. 4A), and extracellularly in association with cell debris (Fig. 4B). The substructure of several individual particles is seen in Fig. 4C–F. Some sections clearly showed the outer coat with peripheral subunits or spikes (Fig. 4C). The coat was separated from the core by a layer of low density (Fig. 4C). A particle was occasionally seen in the process of budding from the plasma membrane (Fig. 4D). The base of a few long particles presented an appearance suggestive of the axial pore seen in the negatively stained particles (Fig. 4E). Cross-sections of these particles were also seen (Fig. 4C and F).

MORPHOLOGY OF SRCD VIRUS

Negatively stained concentrates of medium from SRCD-infected cells 7 days postinoculation revealed particles that were morphologically indistinguishable from IHN-infected cells (Fig. 5A–H). Mean diameter of 25 particles was 90 mμ with a range of 70–100 mμ; mean length was 159 mμ with a range of 120–300 mμ. Ten truncated forms (Fig. 5E) had a mean diameter of 99 mμ and a mean length (distance from the flat surface to the opposite side) of 98 mμ.

The outer coat of these structures is clearly visible in some of the particles (Fig. 5B, C, and H). Particles that are oriented to give a view of the flat end often show evidence of an axial pore (Fig. 5D–F). Sometimes structures were seen in the area of the pore suggesting a short tail (Fig. 5A) and at other times appearing as remnants of host cell membrane that remained adherent to the base of the assumed virus particle (Fig. 5G).

MORPHOLOGY OF OSD VIRUS

Preparations of negatively stained OSD virus from cultures of FHM cells infected for 10 days revealed particles similar to IHN and SRCD viruses (Fig. 6A–E). Mean diameter of 22 particles was 91 mμ with a range of 75–100 mμ; mean length was 181 mμ with a range of 140–220 mμ. Very few truncated forms were seen. The greater mean length, as compared with that of IHN

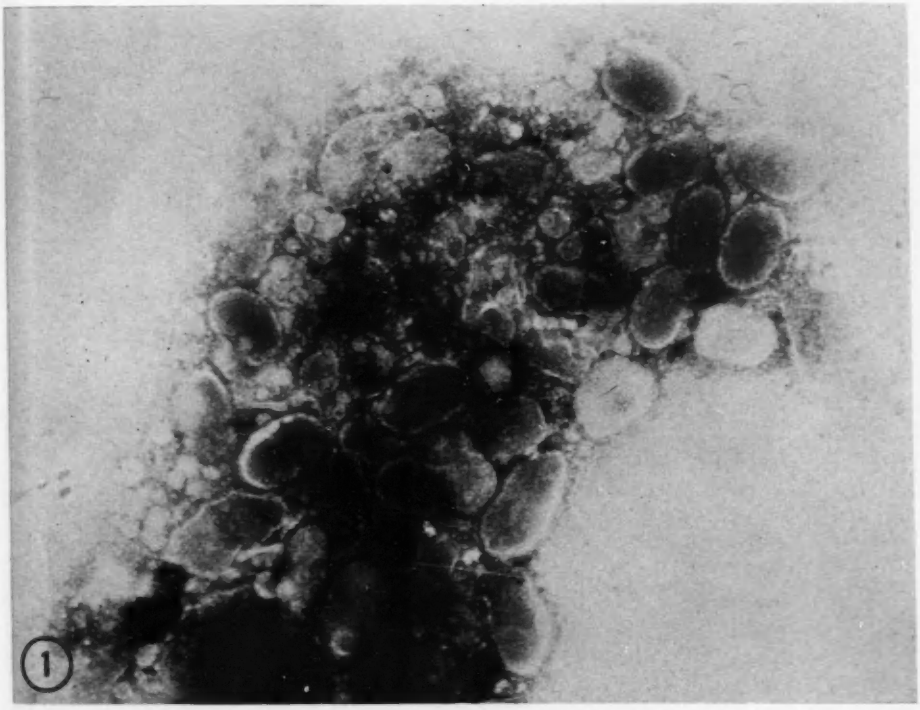

FIG. 1. IHN virus in a negatively stained preparation of material from infected FHM cultures. ×101,000.

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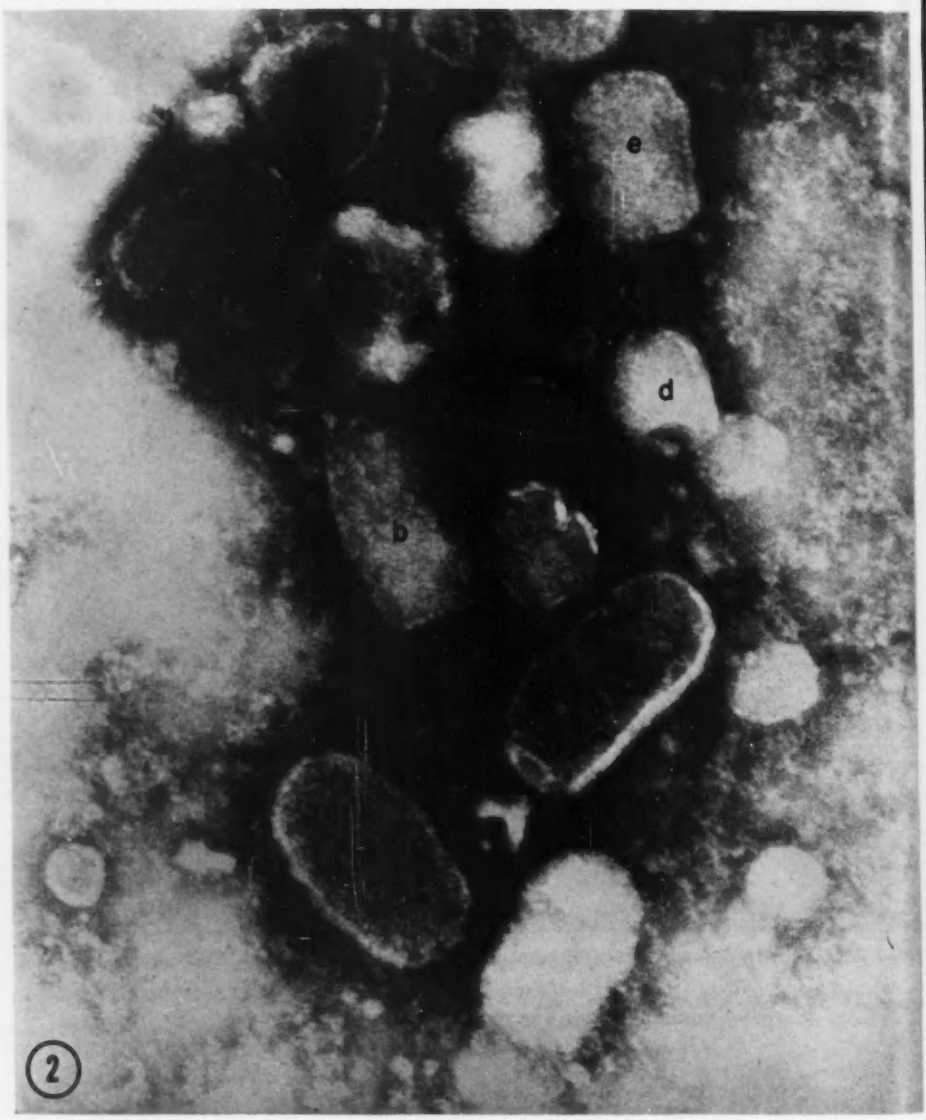


FIG. 2. Negatively stained IHN virus showing variation in shape: (*a*) an ovoid form, (*b*) a bullet-shaped particle, (*c*) a bullet-shaped particle showing a pore at the flat end, (*d*) truncated form of the virus, (*e*) a short form of the virus showing the outer coat. ×224,000.

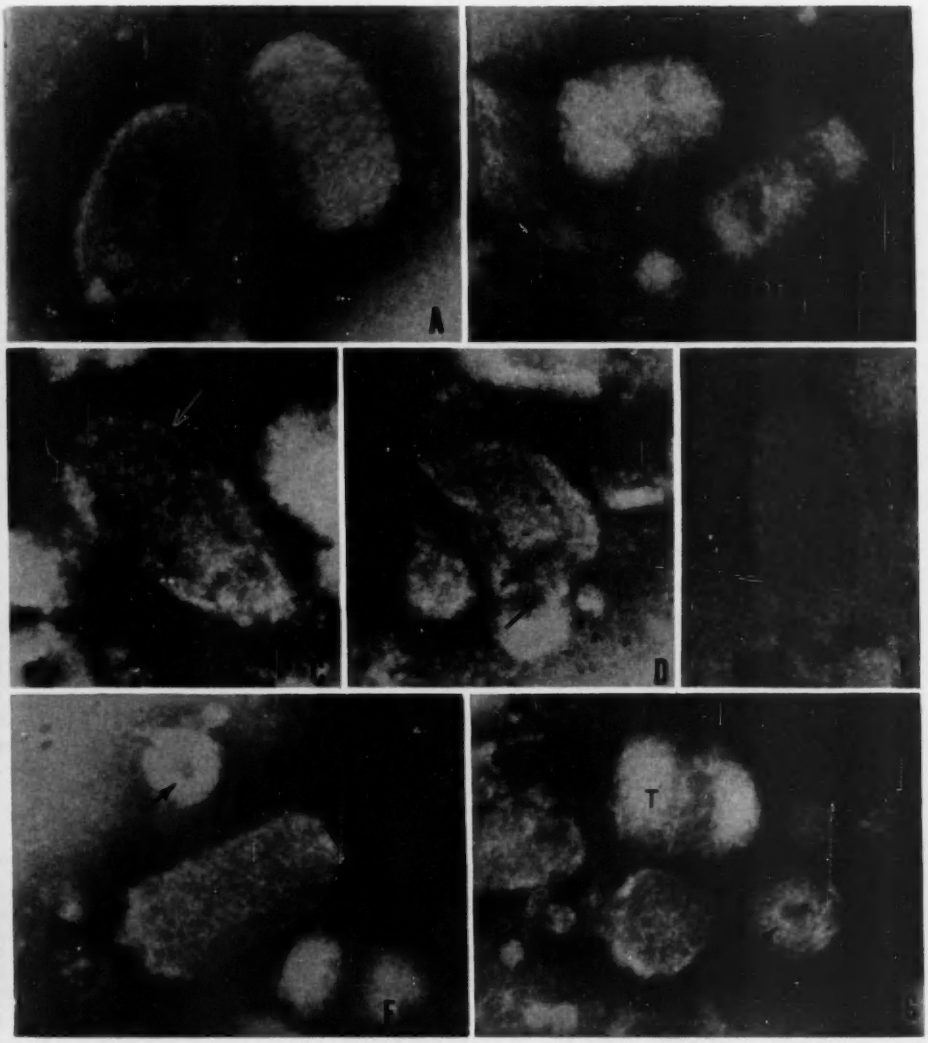
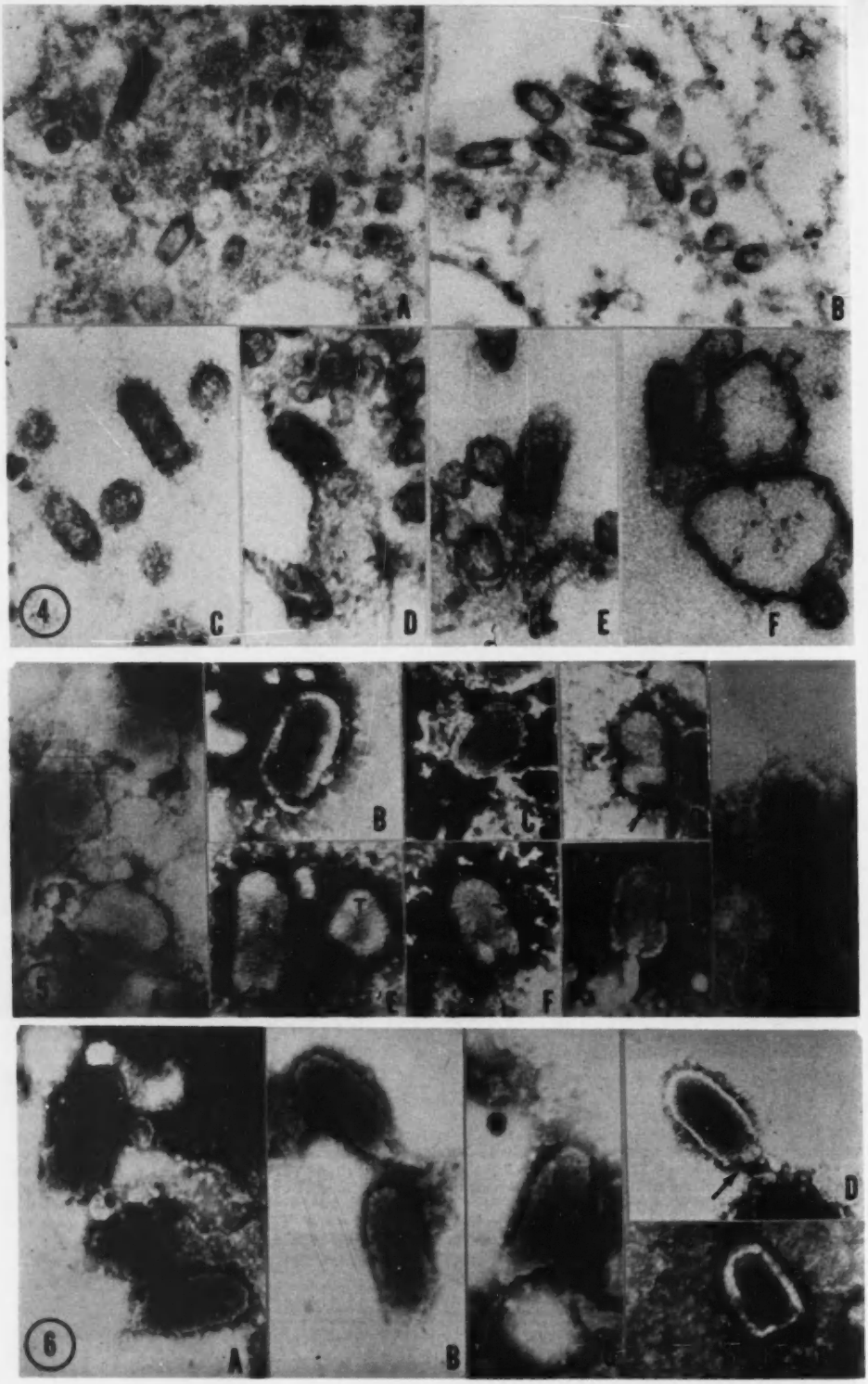


FIG. 3. Negatively stained particles of IHN virus. (A) Two ovoid virus particles. (B) Two bullet-shaped particles. (C) A virus particle showing the outer coat and the internal core separated by a light line suggestive of a membrane. Organization suggestive of subunits is discernible in the outer coat (arrow). A tail-like structure is seen at the base of the particle. (D) A virus particle showing the subunits of the coat and some semblance of the internal structure of the core. A tail-like structure with a hollow center is seen in the region of the pore (arrow). (E) A virus particle showing the outer coat and the core. (F) A bullet-shaped particle showing surface subunits and tail-like structure at the base. A circular profile directly above the bullet-shaped particle is interpreted as a face-on view of the base of another particle showing the pore as a small dark center disc (arrow). (G) A truncated form of the virus (T). A face-on view of the base of another particle shows the pore (arrow). ×224,000.



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or SRCD virus, may be explained by the small number of short forms in the OSD virus preparations. Occasionally, the outer coat and the core were discernable in the negatively stained particles (Fig. 6D). A short tail-like structure extends from the base of the particle in Fig. 6D. Internal cross striations were observed in an occasional damaged virus particle (Fig. 6E).

DISCUSSION

When Amend et al. (1969) first reported the isolation of IHN virus, the morphology of this virus and of SRCD and OSD viruses was not known. Therefore, a comparison of the viruses causing epizootics in salmonid fish was not possible. Negatively stained preparations of IHN virus and thin sections of infected FHM cells clearly showed bullet-shaped particles that were presumed to be IHN virus. Particles with the same morphology were also observed in thin sections of kidney, and spleen from sockeye salmon infected with IHN virus (Amend and Chambers, 1970). A similar bullet-shape morphology was demonstrated from tissues infected with SRCD and OSD viruses in negatively stained preparations. In the above instances, only semipurified preparations were used and a true quantitative relation between physical particles and virus activity was not established. However, since more bullet-shaped particles were seen 7 days after inoculation than after 3 or 4 days and because no similar structures were observed from noninfected tissues, we believe this offers strong presumptive evidence that the particles observed were virus.

IHN virus differs from IPN virus in chloroform or ether sensitivity, in heat sensitivity, and in histopathology. Moreover, IHN is not inhibited by anti-IPN virus serum (Table 1). IPN virus is an icosahedron with a diameter of 65 mμ and is classified as a reovirus (Moss and Gravell, 1969).

IHN virus resembles VHS in sensitivity to chloroform or ether, to low *p*H, and to heat (Jensen, 1965; Zwillenberg and Zwillenberg, 1968). Both

FIG. 4. Sections of FHM cells infected with IHN virus. (A) Virus particles within the cytoplasm of a degenerating cell. ×56,000. (B) Virus particles adhering to extracellular cell debris. ×56,000. (C) A bullet-shaped particle shows spicules extending from the outer coat. A line of low density separates the coat from the core. Striations representing the helical nature of the core are discernible. Four other particles are seen in various circular and elongated profiles. ×101,000. (D) A budding form of a virus particle showing a striated appearance. ×101,000. (E) A bullet-shaped particle with a pore at the base (arrow). ×101,000. (F) An elongated virus particle and possibly a cross-section of another particle associated with cell debris. ×101,000.

FIG. 5. SRCD virus particles in negatively stained preparations of material from infected FHM cultures. (A) Three bullet-shaped particles. The upper two particles show an outer coat. External spicules are seen on the surface of the lower particle. A structure extends from the base of one particle (arrow). (B) A bullet-shaped particle showing subunits of the coat and external spicules. (C) An ovoid particle showing clearly the outer coat and the internal core. (D) A pore is seen at the base of the virus particle (arrow). (E) A bullet-shaped particle with a pore at the base. A truncated form of the virus (T) is at the right. (F) A virus particle showing the pore. (G) A virus particle with material, perhaps of host origin, attached to the base. (H) A virus particle showing the outer coat and a helical internal structure. A–G, ×101,000; H, ×145,000.

FIG. 6. OSD virus particles in negatively stained preparations of material from infected FHM cultures. (A), (B), and (C) show bullet-shaped virus particles with various irregularities at the base. (D) A bullet-shaped virus particle showing the coat, internal core, and a tail-like structure at the base (arrow). (E) A damaged virus particle showing the core with its striated appearance at the edges. ×101,000.

TABLE 1. Comparison of salmonid virus characteristics.

Characteristic[a]	IPN	VHS	IHN	OSD	SRCD
Symmetry	Icosahedral 92 capsomeres	Helical	Helical	Helical	Helical
Size ($m\mu$)	65	65×180	90×158	91×181	90×159
Sensitive to *p*H 3.0	Yes	Yes	Yes	Yes	Yes
Sensitive to chloroform or ether	No	Yes	Yes	Yes	Yes
Inactivated at 60 C for 15 min	No	Yes	Yes	Yes	Yes
Nucleic acid	RNA[b]	RNA	RNA[c]	RNA	RNA[c]
Primary target tissue	Pancreatic	Hematopoietic	Hematopoietic	Hematopoietic	Hematopoietic
Susceptible hosts[d]	Rainbow & brook trout	Rainbow trout	Rainbow trout & sockeye salmon	Rainbow trout & sockeye salmon	Rainbow trout, sockeye, and chinook salmon
Neutralized by anti-IPN virus serum	Yes	No	No[e]	No[e]	No[e]
Neutralized by anti-VHS serum	No	Yes	Unknown	Unknown	Unknown

[a]Unless otherwise indicated, characteristics can be found under References.

[b]Malsberger and Cerini (1965).

[c]Preliminary experiments using 5-Bromo desoxyuridine and 5-Iododesoxyuridine inhibitors suggest that the nucleic acid content is RNA, but these results have not been confirmed.

[d]The list of susceptible hosts includes only those that are useful in comparing the given viruses.

[e]K. Wolf, personal communication.

viruses have an RNA core and both have an affinity for the hematopoietic tissue of trout (Table 1). Also, both viruses appear to be bullet-shaped. However, they present a distinct difference in size, VHS virus being longer and narrower than IHN virus (Zwillenberg et al., 1965). The mean dimensions for VHS virus are 180 × 65 mμ and for IHN virus 158 × 90 mμ. Truncated forms, T particles, were commonly observed in negatively stained preparations of IHN-infected tissue culture concentrates but such particles have not been reported in preparations of VHS virus. Truncated forms of vesicular stomatitis virus (VSV) were described by Huang et al. (1966). They were believed to be incomplete forms of the virus and were often seen after successive passage of the virus at high multiplicity in a single cell line. In our studies IHN virus had undergone about eight passages in FHM cells with no greater than 1:100 dilution at each passage. This may account for the many T particles observed in the preparations of IHN virus.

Amend et al. (1969) indicated some basic differences in the CPE produced by IHN virus in cultures of rainbow trout gonad (RTG-2) cells as compared to the CPE produced by VHS virus in the same cell line. On cover-slip cultures fixed with methanol and stained with May-Grunwald-Giemsa, the first changes observed in IHN-infected cells were increased basophilia of the nuclear membrane and the decrease in density of the chromatins. Later the cells changed from an elongated fibroblast type cell to a spherical shape. These changes were absent in the VHS virus-infected cells. In contrast, RTG-2 cells infected with VHS virus became more elongated and slender, giving a "stringy" appearance to the monolayer. These differences have been confirmed by Jorgenson (State Veterinary Serum Laboratory, Arhus, Denmark, personal communication). It has also been shown that a *p*H of 7.4–7.8 is required for multiplication of VHS virus in RTG-2 cells (Wolf, 1966), whereas IHN virus multiplies well at a *p*H of 7.2.

Differences in the histopathology of the tissues of infected fish were described by Yasutake (1968). He found that fish infected with VHS virus during an epizootic typically showed hemorrhagic lesions in the muscle tissue whereas fish infected with IHN virus showed no lesions of this kind. Amend et al. (1969) found extensive pathological changes in the granular cells of the stratum compactum of fish infected with IHN virus. Such changes were not observed in the tissues of fish infected with VHS virus (Yasutake, 1968).

The assumed morphology of IHN virus, together with its other characteristics (Table 1), indicates that it should be classified as a member of the rhabdovirus group (Jawetz et al., 1966; Melnick and McCombs, 1966). The differences in size, CPE in tissue culture cells, and histopathology between IHN and VHS viruses indicate that they are sufficiently different to be included as separate viruses within this group. Serological studies are needed to determine whether they are related antigenically.

The viruses of OSD and SRCD are indistinguishable from IHN virus in the following respects: morphology, RNA core, *p*H-, ether-, and heat-sensitivity, and in susceptibility of hematopoietic tissue as the primary target tissue (Amend

1968; Parisot, 1967; Parisot et al., 1965; Wingfield et al., 1969). Although OSD virus was described as ether-resistant in an early report (Parisot et al., 1965), later studies have shown it to be ether-sensitive (Wingfield et al., 1969). In addition, all three viruses appear to be bullet-shaped. Measurements of electron microscope images of negatively stained particles from infected cells were similar and none of the differences were considered significant. Also, the CPE produced by IHN, OSD, and SRCD in FHM and RTG-2 tissue cultures was indistinguishable (Amend et al., 1969) and the extracellular virus reached approximately the same titer in each case (K. Wolf, personal communication). Finally, all three viruses have been shown to infect rainbow trout and sockeye salmon (Amend et al., 1969; Parisot et al., 1965). However, chinook salmon are susceptible to infection with SRCD virus, but susceptibility of chinook salmon to IHN and OSD viruses has not been conclusively determined. Coho salmon (*Oncorhynchus kisutch*) are refractory to all three virus diseases (Amend et al., 1969; Parisot et al., 1965).

IHN, SRCD, and OSD viruses are indistinguishable in most respects and may be closely related or perhaps separate isolates of the same virus. Supportative evidence has been presented by McCain (MS, 1970), who reported similar bullet-shaped particles of OSD virus and showed by cross-plaque neutralization that IHN, OSD, and SRCD viruses were serologically related. However, further serological studies are needed to determine whether they are antigenically identical and should be regarded as a single member of the rhabdovirus group, or whether any one of them is antigenically distinct from the others and should be included as a separate member(s) of this group.

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Influence of Photoperiod on Smolt Development and Growth of Atlantic Salmon (*Salmo salar*)

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Atlantic salmon (*Salmo salar*) subjected in the spring to a constant photoperiod of 13 hr light/day or to simulated natural photoperiod (increasing day length) smoltified and grew rapidly before and after entering sea water. Others subjected to the reciprocal (complement) of natural photoperiod (decreasing day length in the spring) had the silvery color of smolts and grew rapidly but, unlike smolts, developed high condition factors while in fresh water. After they were transferred to sea water these fish ate less, grew more slowly, and had lower efficiencies of food conversion than those subjected to natural and constant photoperiod.

Salmon experiencing reciprocal photoperiod had decreased sensitivity to external stimuli, suggesting reduced thyroid activity. Histological studies in June showed no differences in the appearance of the thyroid glands, suggesting no difference in thyroid activity between groups in natural and reciprocal photoperiod. Nor was there any difference in plasma osmotic and chloride levels between natural and reciprocal photoperiod groups, either in routinely active or fatigued fish.

It is suggested that photoperiod acts through the endocrine system to affect growth patterns during and after smolting and possibly certain aspects of the smolting process itself. Effects of photoperiod on smolting and growth of salmon are discussed in connection with other cyclical changes in salmonid physiology known to be influenced by photoperiod.

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INTRODUCTION

FISHES IN NORTH TEMPERATE REGIONS have seasonal growth cycles and usually show maximum growth rates during the spring and early summer — a period of abundant food supply and of increasing temperature and day length. Although temperature and food supply are undoubtedly important factors in promoting growth, studies by Gross et al. (1965) emphasize the importance of photoperiod on the growth of green sunfish (*Lepomis cyanellus*). They demonstrated that fish grew slightly better with a constant 16-hr day than with an 8-hr one and that gradually increasing day length enhanced growth considerably, whereas decreasing day length depressed it.

The Atlantic salmon, *Salmo salar*, has a remarkable growth rate after smolt development and is, therefore, a good subject for studies of the effect of photoperiod. Smolts weighing 25–50 g at the time of their seaward migration commonly reach 1.5–3 kg by the end of their 1st year at sea. Moreover, the

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smolting process in anadromous fishes and a number of physiological and behavioral changes that comprise the process are probably triggered by changing photoperiod (Hoar, 1965). Smoltification preadapts the salmon for the transition between river and marine environments and for its marine life. It is likely that some aspects of smolting set the biochemical stage for metabolic and growth patterns in the marine environment and that any disruption of smolting would affect these growth patterns. This laboratory study was carried out to show the effects of manipulation of photoperiod on the smolting process and on growth rates of Atlantic salmon during and after smolting.

MATERIALS AND METHODS

Experiments were conducted in 1967, 1968, and 1969. Each year, separate new groups of salmon were subjected to different photoperiods, beginning in the spring, while maintaining similar temperature, salinity, and feeding conditions among groups. The photoperiod regimes were: simulated natural, i.e., increasing day lengths from late December to late June and decreasing thereafter; reciprocal or complement of natural photoperiod, i.e., decreasing day length until late June and increasing thereafter; and constant day length, 13 hr light/day. The 1967 experiments were exploratory. They yielded some useful data and indicated more profitable ways to manipulate photoperiod, temperature, and salinity in subsequent experiments. The crucial experiments were done in 1968 and conclusions from these were verified in 1969.

Fish-Holding Conditions

Salmon were held in cylindrical, fiberglass tanks, 1.83 m diam, filled to a depth of 0.6 m. Flow rates of fresh or sea water were maintained at about 15 liters/min. A submersible electric pump at the periphery of each tank enhanced self-cleaning by carrying waste food and feces to a center drain. The water supply together with the submersible pump maintained a current speed of about 14 cm/sec near the periphery of the tank. This current elicited a positive rheotactic response by the salmon and improved feeding conditions by dispersal of the fish throughout the tank and by imparting movement to the food.

Each tank was enclosed in a light-proof compartment. A two-bulb fluorescent light fixture with daylight-type tubes rated at 92 w was centered 94 cm over the water surface of each tank. Lights were controlled by automatic timers, preset to turn the power on or off at specified times. Lights went on or off suddenly; no attempt was made to simulate dawn or dusk conditions. Light intensity at the water surface was 150 ft-c. In the water at a point 30 cm from the periphery it was 35 ft-c 10 cm below the surface, and 28 ft-c at a depth of 46 cm. Towards the center of the tank, 60 cm from the perimeter, the light intensity was 43 ft-c 10 cm below the surface and 31 ft-c at a depth of 46 cm.

1967 Experiment

Hatchery-reared yearling salmon were brought to the laboratory on April 7 and held in fresh water at 10 C with natural photoperiod. Three groups of 80 smolts were placed in separate tanks in fresh water on April 20 after measurement of individual fish length and weight. One group was continued under natural photoperiod; constant and reciprocal photoperiod regimes were started for the other two groups. Salinities were gradually raised from 0 (fresh water) to 30‰ between April 21 and 28. Temperature was raised gradually from 10 to 15 C between April 20 and May 5 and held at 15 ± 1 C thereafter. The timetable for manipulation of photoperiod, temperature, salinity, and distribution of fish is given in Fig. 1.

The fish were fed a mixture of ground beef liver, cod fillets, and Purina Trout Chow (42.5, 42.5, and 15% by weight respectively). They were fed to repletion twice daily on weekdays and once daily on weekends and holidays. Food was introduced in small enough lumps so that the fish could take them with little being wasted. Food was offered until the fish allowed it to sink to the bottom of the tank, which was taken as an indication that they were satiated. Feeding times were

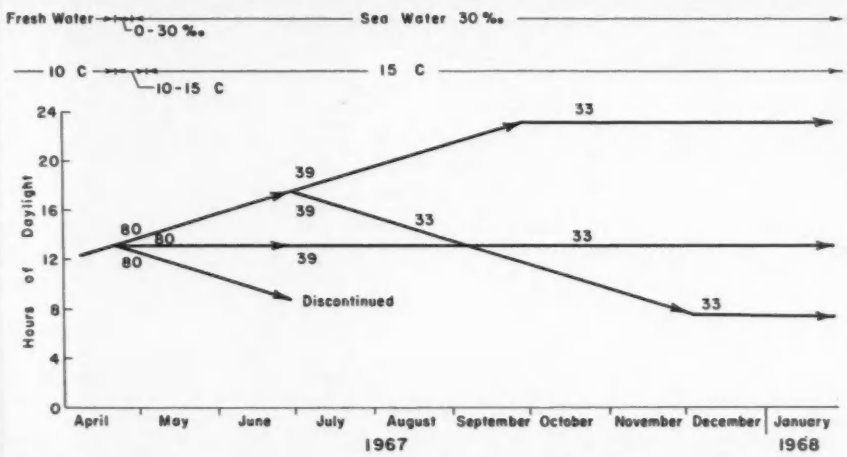

FIG. 1. Timetable for manipulation of photoperiod, temperature, salinity, and distribution of fish in 1967 experiment. The increasing light regime was designed to simulate natural photoperiod at St. Andrews, N.B. (45°N lat); the decreasing photoperiod was the reciprocal (complement) of natural photoperiod. On June 29 there was a redistribution of fish and establishment of new photoperiod regimes for some of the fish. The reciprocal photoperiod group was discontinued. The increasing photoperiod group was divided by random numbers into two groups, one to continue on increasing day length until September 29 and the other to have decreasing day length until December 2. The constant photoperiod group was divided into two groups; one was continued on the same photoperiod and the other discarded. Day length was changed by increments or decrements of 15 min every 4th day. Numbers of fish in groups at different times are indicated.

arranged so that the lights had been on for an hour or more before feeding. Records were kept of the weight of food given so that mean efficiency of food conversion could be determined as follows:

$$\frac{\text{gain in wet weight of the group during the period}}{\text{wet weight of food given during the period}} \times 100.$$

Owing to the type of food and method of feeding, conversion efficiencies are minimum values. Some food was uneaten and lost via the tank drain. No attempt was made to recover this food or to account for it when calculating conversion efficiency.

1968 EXPERIMENT

This experiment differed from the one in 1967 in the following respects: (1) reciprocal and constant photoperiod regimes were started in early March rather than late April; (2) observations on the reciprocal photoperiod group were continued after late June, not discontinued as in 1967; and (3) fish were held in fresh water until May 15 and then subjected to a sudden increase to 30‰ salinity during 24 hr rather than experiencing a gradual increase in salinity during 7 days beginning on April 20, the day photoperiod regimes were started in 1967 (Fig. 2).

Hatchery-reared underyearling salmon were brought to the laboratory on November 16, 1967, held in fresh water at 10–12 C, and subjected to natural photoperiod during the winter. Three groups of 30 presmolts to be used in the growth experiment were placed in separate tanks of fresh water on March 8, 1968, after measurement of individual fish length and weight. Three groups of 40 fish to be used for determination of plasma osmotic and chloride concentrations were similarly treated on March 8 and placed in separate tanks of fresh water. Temperature was raised from 12 to 15 C between March 10 and April 15 and held at this level (±1 degree C) until the end of the experiment on December 6. Salinity was increased from 0 to 30‰ on May 15 and maintained at this level (±1‰) until October 22. From October 22 to December 6 the fish were held in fresh water.

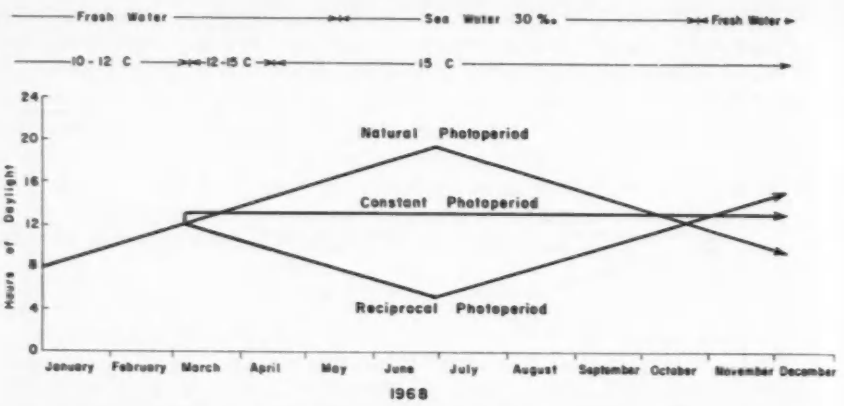

FIG. 2. Timetable for manipulation of photoperiod, temperature, and salinity in 1968 experiment. Day length was changed by increments or decrements of 15 min every 4th day. There were 30 fish in each group throughout the experiment.

Two tanks of fish were maintained under each photoperiod regime, one for growth measurement, the other for determination of plasma osmotic and chloride concentrations. The fish in the holding tanks swam at a speed of about one-half body length/sec. This is described as routine activity in contrast to strenuous exercise, which was imposed before blood sampling in the 1969 experiment by forcing the fish to swim for 1 hr at about 3 body lengths/sec in a tunnel respirometer like that described by Farmer and Beamish (1969).

Blood samples were taken by cutting off the caudal peduncle and collecting the blood that issued from the caudal artery in a heparinized centrifuge tube. Blood was centrifuged at 2800 rpm for 3 min. Total osmotic concentration was determined in .25-ml samples of plasma using an Advanced Osmometer, Model 31LAS. Plasma chloride was estimated in .1-ml samples using a Buchler–Cotlove chloridometer, Model 4-2008. Sampling covered the periods before, during, and after smoltification and before and after the fish entered sea water. The diet and feeding procedure were the same as in 1967.

1969 EXPERIMENT

This experiment was designed to repeat parts of the one done in 1968. Only two photoperiod regimes were used, natural and reciprocal. Fish of the same age-class and stock were brought to the laboratory at the same time of year and kept under similar temperature and photoperiod conditions during the winter as those used in 1968. The timetable for manipulation of photoperiod and elevation of temperature and salinity was similar to that used in 1968 (Fig. 2). Duplicate lots of fish for plasma osmotic and ionic measurements and for growth studies were not kept in 1969; instead, some of the fish from the growth experiments were used for osmotic and ionic measurements. The diet in 1969 was pelleted Purina Trout Chow.

COLLECTION OF GROWTH DATA

Individuals were weighed to the nearest gram and measured to the nearest millimeter (fork length) at approximately monthly intervals. No food was given on days fish were measured. The fish were anesthetized (about six at a time) in tertiary amyl alcohol of such strength (5–10 ml/liter) that they were immobilized quickly. After measurements were made, fish were put in clean water where recovery to the breathing stage was usually made in 5 min.

Data processing was done by computer analysis as described by Saunders and Henderson (MS, 1969).

Thyroid Studies

Assessments of thyroid activity based on histological study of follicular cell height were made in June 1969 for the increasing and decreasing photoperiod groups following the methods outlined by Eales (1963). Tissues surrounding the ventral aorta were fixed in Bouin's formol–picric –acetic acid fixative. Sections 7–10 μ thick were stained in Mallory's triple stain. The criteria for thyroid activity were follicular cell height and staining reaction of the stored colloid (Woodhead and Woodhead, 1965).

RESULTS

1967 Experiment

Growth rates–condition factor — Mean lengths of fish in the three groups did not diverge during the period April 20–June 29 (Table 1). The mean weight in the reciprocal photoperiod group was significantly greater ($P < 0.01$)

Table 1. Length, weight, and condition factor in relation to photoperiod, April–June 1967. Salinity raised from 0 to 30‰ between April 21 and 28 and held at 30±1‰ thereafter. Temperature at first measuring date 10 C and 15 C thereafter.

Photoperiod regime	No. fish	Mean fork length (*cm*)	Mean wt (g)	Condition factor $\left(K = \frac{100 \times W}{L^3}\right)$
		Apr. 20		
Increasing (natural)	80	15.3±.097[a]	35.4±.707[a]	0.974±.0049[a]
Decreasing (reciprocal)	80	15.3±.087	35.0±.602	0.978±.0050
Constant	80	15.3±.105	35.0±.814	0.964±.0054
		May 25		
Increasing (natural)	80	19.3±.108	72.7±1.363	1.004±.0052
Decreasing (reciprocal)	80	19.2±.098	73.3±1.112	1.026±.0057
Constant	80	19.2±.114	70.6±1.412	0.981±.0056
		June 29		
Increasing (natural)	78	23.4±.143	127.7±2.608	0.988±.0056
Decreasing (reciprocal)	80	23.2±.143	137.4±2.672	1.089±.0072
Constant	77	23.2±.152	125.1±2.860	0.995±.0055

[a]Standard error of the mean.

by late June than those of the other two groups. There was essentially no difference between the natural and constant photoperiod groups in respect to length, weight, or condition factor. Condition factor (Table 1) for the reciprocal photoperiod group was significantly higher ($P < 0.01$) in June than those of the other two groups. Under natural conditions, smolts have a low condition factor and this is one of the characteristics that distinguish smolts from parr (Hoar, 1939). Therefore, it appears that the reciprocal photoperiod may have affected the smolting process.

There were no marked differences in growth rate among groups for the first 3 months after redistribution of the original natural photoperiod group

into continued increasing and decreasing photoperiod groups (Table 2). By October 12 there were small differences in mean length, weight, and condition factor among the three groups; the increasing photoperiod fish were larger, but not significantly so, than those in the other groups. This trend continued and by the end of the experiment the increasing photoperiod fish were significantly longer and heavier than the other two groups ($P < 0.01$ for length and $P < 0.025$ for weight). Condition factors during the period June 29–January 18 tended to be slightly higher in the increasing photoperiod group

TABLE 2. Length, weight, and condition factor in relation to photoperiod, July 1967–January 1968. The increasing photoperiod group from Table 1 was divided in two on June 29. One half was continued on increasing photoperiod and the other half subjected to decreasing photoperiod. Half of the original constant photoperiod group (Table 1) were continued on constant photoperiod. The other half and all of the decreasing photoperiod group were discarded. Salinity held at 30±1‰, temperature held at 15 C.

Photoperiod regime 1967–68	No. fish	Mean fork length (*cm*)	Mean wt (*g*)	Condition factor $\left(K = \frac{100 \times W}{L^3}\right)$
		June 29		
Increasing	39	23.3±.185[a]	126.7±3.490[a]	0.989±.0075[a]
Decreasing	39	23.4±.220	128.7±3.916	0.987±.0085
Constant	39	23.3±.244	128.8±4.776	0.997±.0092
		Aug. 3		
Increasing	39	27.6±.258	232.9±7.505	1.096±.0110
Decreasing	33	27.1±.295	213.1±6.900	1.057±.0068
Constant	39	27.1±.330	220.4±9.148	1.078±.0108
		Sept. 7		
Increasing	39	30.8±.300	346.3±11.431	1.164±.0094
Decreasing	33	30.6±.374	331.7±12.312	1.135±.0134
Constant	39	30.3±.366	326.0±12.888	1.144±.0104
		Oct. 12		
Increasing	33	33.8±.334	478.4±17.518	1.224±.0126
Decreasing	33	33.2±.420	456.4±17.644	1.220±.0171
Constant	33	32.7±.398	441.4±18.085	1.243±.0121
		Dec. 7		
Increasing	33	37.8±.337	675.8±25.115	1.232±.0149
Decreasing	33	36.3±.504	603.5±26.093	1.227±.0232
Constant	33	35.5±.546	570.4±33.086	1.224±.0377
		Jan. 18		
Increasing	33	40.2±.424	807.4±30.679	1.225±.0137
Decreasing	33	38.1±.570	704.7±33.131	1.222±.0277
Constant	33	37.6±.654	701.6±37.659	1.371±.0152

[a]Standard error of the mean.

than in the other groups. These observations are in agreement with those of Gross et al. (1965), who found that increasing day length promoted growth of green sunfish.

Food conversion efficiency — Efficiencies of food conversion during the period April 20–June 29 were nearly identical in the increasing and decreasing photoperiod groups (Table 3). Efficiencies in the constant photoperiod group were

TABLE 3. Food conversion efficiency of Atlantic salmon in relation to photoperiod in 1967 experiment. Values expressed as mean percentages. First part of the table (April 20–June 29) corresponds with Table 1; second part (June 30–January 18) corresponds with Table 2. Numbers of fish are the same as listed in Tables 1 and 2 for respective dates.

Period (*1967*)	Photoperiod		
	Increasing (%)	Decreasing (%)	Constant (%)
Apr. 20–May 25	31	30	32
May 26–June 29	22	21	26
		↓ Discontinued	↓
	Increasing	Decreasing	Constant
June 30–Aug. 3	23	23	22
Aug. 4–Sept. 7	20	21	22
Sept. 8–Oct. 12	19	17	19
Oct. 13–Dec. 7	18	15	15
Dec. 8–Jan. 18, 1968	16	18	14

higher than those in the other two groups. After redistribution of the fish on June 30, efficiencies varied little among the three groups during the rest of the experiment. The differences in growth rate among the three groups during the period June 30–January 18 were, therefore, not due to different efficiencies of food conversion but to differences in food intake.

Coloration — In this and subsequent experiments, all groups of fish developed the typical silvery color of smolts while in fresh water and showed no obvious color changes after the change from fresh to sea water.

1968 EXPERIMENT

Growth rates–condition factor — While all fish were in fresh water, those subjected to decreasing day length gained weight faster than those in increasing and constant day length (Fig. 3). There were insignificant differences in length among the three groups. Therefore, the faster growth in weight of the fish experiencing reciprocal photoperiod is clearly reflected in condition factor (Fig. 4). Mean condition factor of the reciprocal photoperiod fish was consistently higher than those of the other groups.

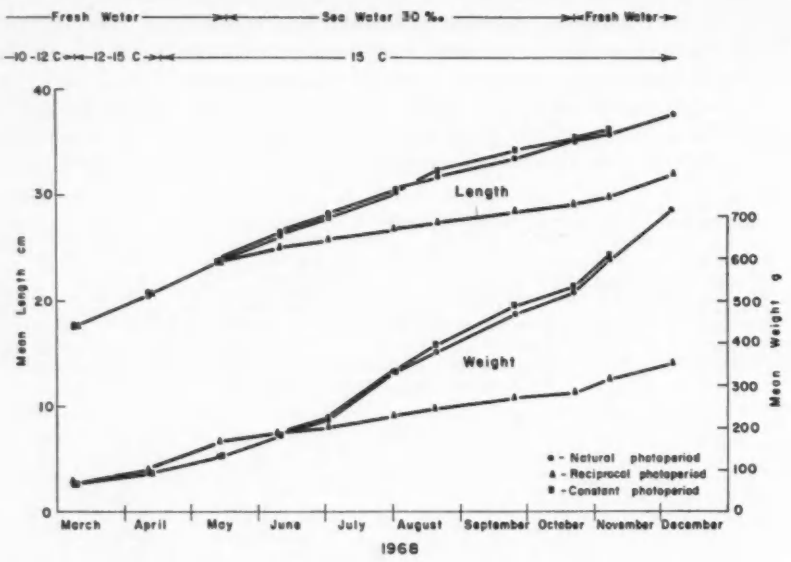

FIG. 3. Mean length and weight of salmon in relation to different photoperiod regimes in 1968. Constant photoperiod group discontinued after November 7.

After the fish entered sea water, the reciprocal photoperiod group ate less food than each of the other groups. Growth in length and weight of fish in the reciprocal photoperiod group declined sharply and fell below the rates of the other two groups, which differed little from one another during the rest of the experiment (Fig. 3). By October, the lengths and weights of the natural and constant photoperiod fish exceeded those of the reciprocal photoperiod group by about 5 cm and 250 g respectively.

After entry into sea water, condition factor of the reciprocal photoperiod fish maintained the high level established in fresh water (Fig. 4). Condition factors of the natural and constant photoperiod fish, which had declined while the fish were in fresh water, rose steadily after they entered sea water and exceeded that of the reciprocal photoperiod fish from September onward.

Food conversion efficiency — Food conversion efficiency was similar among the three groups while they were in fresh water (Table 4). Conversion efficiency of all groups declined after the fish entered sea water. In sea water, efficiencies for the reciprocal photoperiod group were consistently lower than those for the other two groups that had similar efficiencies. The return to fresh water stimulated growth in weight and resulted in increased efficiency in all groups.

Osmotic concentrations — Plasma osmotic and chloride levels were measured in blood taken from fish that had been routinely active in the holding tanks. During the smolting period and prior to the change from fresh to sea water, there were no significant differences in osmotic and chloride levels among the

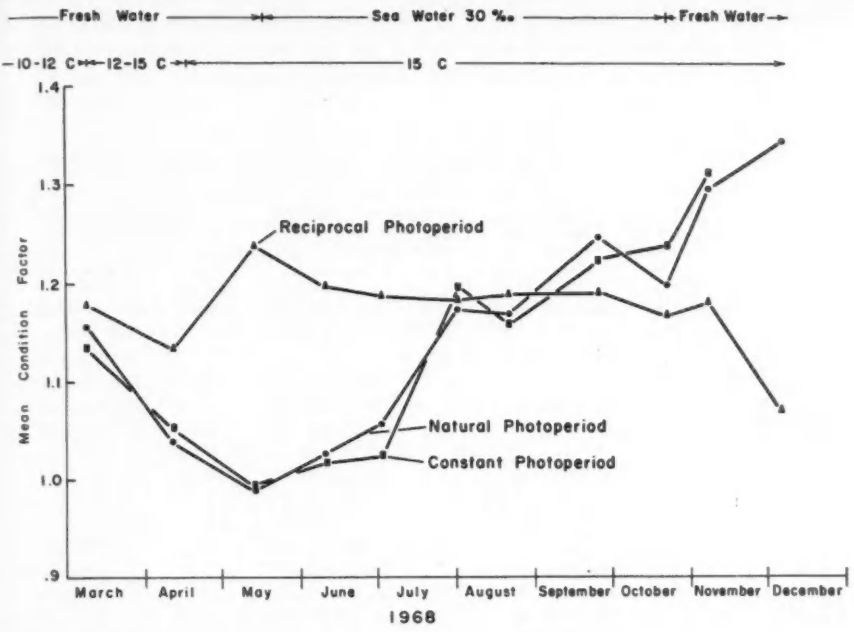

FIG. 4. Mean condition factor of salmon under different photoperiod regimes in 1968. Constant photoperiod group discontinued after November 7.

TABLE 4. Food conversion efficiency of Atlantic salmon in relation to photoperiod in 1968 experiment. Values expressed as mean percentages. Fish were in fresh water during the first period, in sea water during the next six, and in fresh water during the last two periods. Each value based on about 30 fish.

Period (*1968*)	Photoperiod		
	Reciprocal (%)	Constant (%)	Natural (%)
Apr. 11–May 13	27	24	26
May 15	Salinity increased from 0 to 30‰		
May 13–June 10	09	18	18
June 10–July 2	10	14	16
July 2–Aug. 1	14	25	21
Aug. 1–Aug. 21	13	17	15
Aug. 21–Sept. 25	12	16	18
Sept. 25–Oct. 22	08	13	14
Oct. 22	Salinity decreased from 30 to 0‰		
Oct. 22–Nov. 7	20	21	21
Nov. 7–Dec. 6	14	–	20

three groups of fish (Tables 5, 6). After the increase in salinity, there were rises in osmotic and chloride levels in all groups but no clear-cut differences among groups that might be correlated with different photoperiods and resulting differences in growth rate.

TABLE 5. Plasma osmotic concentrations of salmon at various times in relation to photoperiod.

Sampling date (*1968*)	Increasing–decreasing (natural) photoperiod		Decreasing–increasing (reciprocal) photoperiod		Constant photoperiod (*13 hr/day*)	
	No. in sample	Osmotic concn (*milliosmols/kg*)	No. in sample	Osmotic concn (*milliosmols/kg*)	No. in sample	Osmotic concn (*milliosmols/kg*)
Feb. 6–27	8	318.9±2.16[a]	–	– –	–	– –
Apr. 3–8	4	313.5±1.85	5	309.2±2.20[a]	4	315.2±4.50[a]
30	5	316.8±2.56	5	315.4±1.75	5	315.6±3.40
May 14	5	321.8±3.95	5	313.8±1.98	5	312.2±3.97
15			Salinity changed from 0 to 30‰			
16	4	323.8±4.63	5	337.8±3.89	4	336.0±5.82
21	–	– –	5	321.6±3.57	–	– –
28	5	325.2±3.67	4	323.0±2.61	5	315.4±2.71
June 18	5	322.4±4.34	5	315.8±1.85	5	321.2±2.33
July 4	5	332.6±2.46	5	323.6±2.87	5	322.2±5.31
Aug. 2	5	330.0±3.05	5	321.4±2.84	5	323.8±1.07
22	4	331.8±1.11	4	323.8±2.98	4	327.0±1.08

[a]Standard error of the mean.

TABLE 6. Plasma chloride concentrations of salmon at various times in relation to photoperiod.

Sampling date (*1968*)	Increasing–decreasing (natural) photoperiod		Decreasing–increasing (reciprocal) photoperiod		Constant photoperiod (*13 hr/day*)	
	No. in sample	Chloride concn (*mEq/liter*)	No. in sample	Chloride concn (*mEq/liter*)	No. in sample	Chloride concn (*mEq/liter*)
Apr. 30	5	137.3±3.8[a]	5	132.5±2.9[a]	5	130.4±3.5[a]
May 14	5	138.1±0.9	5	134.7±1.3	5	137.1±1.1
15			Salinity changed from 0 to 30‰			
16	5	141.3±0.7	5	145.7±2.8	5	144.2±1.7
21	–	– –	5	142.7±1.1	–	– –
28	5	143.0±0.5	4	141.8±0.5	5	142.7±0.9
June 18	5	143.8±1.5	5	142.3±0.8	5	142.9±1.1
Aug. 2	5	141.6±1.5	5	141.6±0.6	5	138.5±2.2
22	4	145.0±0.5	4	141.2±1.4	4	143.1±0.6

[a]Standard error of the mean.

Excitability — There was a marked difference in the sensitivity to external stimuli. Fish exposed to natural and constant day length were highly excitable and difficult to catch in a dipnet; the reciprocal photoperiod fish were much

less disturbed by our movements near the holding tank and could be netted easily.

1969 Experiment

Growth rates–condition factor — This experiment, with the same timetable for manipulation of temperature, photoperiod, and salinity as the one in 1968, confirmed the findings of the 1968 experiment. During the freshwater phase of the experiment, the reciprocal photoperiod fish grew faster in weight than those in natural day length (Fig. 5). This faster growth was reflected

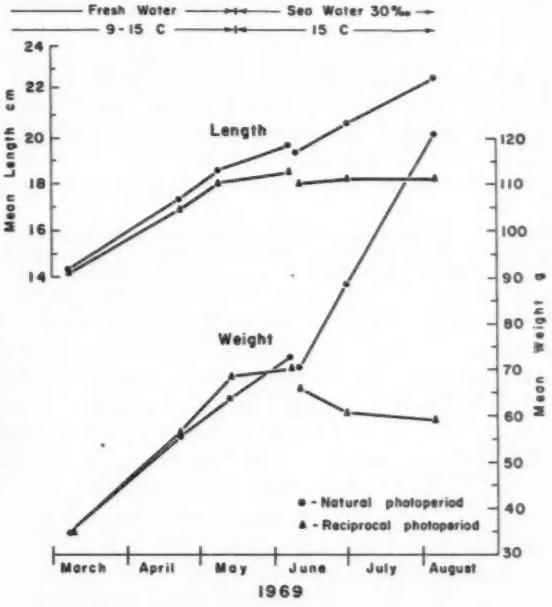

FIG. 5. Mean length and weight of salmon under natural and reciprocal photoperiod regimes in 1969. Ten fish were removed from each group on June 9 for blood and thyroid studies. New mean lengths and weights were calculated for the remaining fish and the experiment continued.

in condition factor (Fig. 6). As in the 1968 experiment, growth in length and weight of the reciprocal photoperiod fish was sharply reduced when they entered sea water (Fig. 5). Condition factor fell in reciprocal photoperiod fish and rose in the natural photoperiod group after entry into sea water (Fig. 6). Food conversion efficiency was not measured in this experiment but it was observed that food intake by the reciprocal photoperiod fish decreased abruptly when they entered sea water.

Excitability — As in 1968, the fish experiencing reciprocal photoperiod were much less sensitive to external stimuli than those on natural photoperiod.

Osmotic concentrations — Since in the 1968 experiment there was no difference among plasma osmotic and chloride concentrations attributable to photoperiod, comparisons were made in 1969 for these parameters between routinely

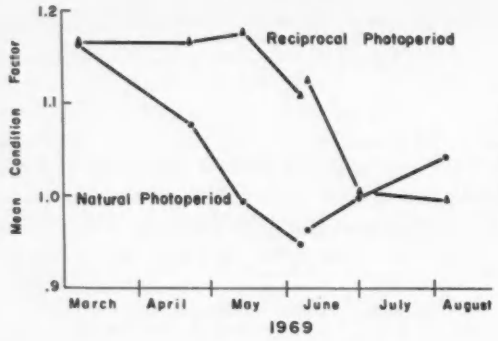

FIG. 6. Mean condition factor of salmon under natural and reciprocal photoperiod regimes in 1969. Ten fish removed from each group on June 9 for blood and thyroid studies. New condition factors were calculated for the remaining fish and the experiment continued.

active and strenuously exercised fish (Table 7). Rao (1969) found that exercised rainbow trout, *Salmo gairdneri*, had higher plasma osmotic concentrations than resting fish. Strenuous exercise resulted in elevation of both osmotic and chloride levels over those for routinely active salmon. However, these data do not indicate any difference attributable to photoperiod regime.

TABLE 7. Plasma osmotic and chloride levels measured for individual routinely active and strenuously exercised salmon smolts under different photoperiod regimes on June 9, 1969. Because of the small size of the fish, it was not always possible to get enough plasma for both osmotic and chloride measurements.

	Routinely active		Exercised	
Photoperiod regime	Osmotic concn (*milliosmols/kg*)	Chloride concn (*mEq/liter*)	Osmotic concn (*milliosmols/kg*)	Chloride concn (*mEq/liter*)
Natural photoperiod	328	–	336	–
	309	144.9	340	–
	314	–	351	–
	310	138.9	343	–
Reciprocal photoperiod	322	145.9	375	–
	322	147.2	351	159.3
	322	145.9	–	149.3
	326	–	–	156.4
	319	142.8		

Thyroid studies — The samples taken from natural and reciprocal photoperiod groups on June 9 revealed no histological differences between the two groups. Thyroid follicles from both groups had low epithelial cells and were well filled with dense colloid material, both indicative of low thyroid activity.

DISCUSSION

SMOLTIFICATION DISTURBED BY RECIPROCAL PHOTOPERIOD

In spite of the differences in timetables for manipulation of photoperiod, temperature, and salinity among the three experiments, the fish that exper-

ienced reciprocal photoperiod had consistently higher rates of growth in weight and consequently higher condition factors during late April and May than fish exposed to natural or constant photoperiod. The higher condition factor in the spring together with the dramatic suppression of growth rates of reciprocal photoperiod fish in sea water suggest that reciprocal photoperiod disturbed the smolting process. Subjecting the fish to a reciprocal photoperiod starting as late as April 21, 1967, was sufficiently early to affect length–weight relations during the smolting period.

Since growth rates, efficiencies of food conversion, sensitivity to external stimuli, and osmotic regulation were similar in the constant and natural photoperiod groups in the 1968 experiment, it is suggested that the smoltification process was started by early March and was sufficiently well established by that time that the initiation of a constant photoperiod regime (13 hr light/day) did not upset it. However, a decreasing photoperiod regime acting from March through June in the 1968 and 1969 experiments appeared to disturb certain aspects of the smolting process and to alter subsequent growth patterns.

Thyroid Function and Excitability

Most species of anadromous fishes, including Atlantic salmon, show increased thyroid activity at the time of migration, and this increase might be a factor causing induction of migration (Baggerman, 1960a). Moreover, Baggerman says that salmon smolts are more sensitive to external stimuli than are parr and suggests that this may be owing to greater thyroid activity in smolts. If sensitivity to external stimuli is a characteristic of smolts, then the lack of such sensitivity in the salmon subjected to reciprocal photoperiod is further evidence that they did not become normal smolts.

The histological evidence did not support the hypothesis that the differences in sensitivity to external stimuli between reciprocal and natural photoperiod groups were due to different thyroid activity. However, histological methods may be unreliable for measuring thyroid activity. Drury and Eales (1968) report a discrepancy between the results of histological and radiochemical measurements of thyroid activity in brook trout, *Salvelinus fontinalis*, and give evidence that radiochemical methods are more reliable.

Feeding Appetite and Growth Patterns

The most reasonable explanation for the striking differences in growth rate in relation to salinity is that appetite and efficiency of food conversion are affected by photoperiod. Saunders and Henderson (MS, 1969) show that there are seasonal changes in feeding and growth of postsmolt salmon in relation to salinity. During the spring and early summer, food conversion efficiencies were similar and high among groups in water of 15 and 30‰ salinity and in fresh water. Differences in growth were, therefore, attributable to differences in appetite and food consumption rather than to efficiencies of food conversion. Later in the season efficiencies of food conversion were lower for 30‰ salinity

than for 15‰ and freshwater groups. The authors attribute this to seasonal changes in physiology of salmon, which accords with Hoar's (1965) hypothesis to account for a changing physiology of salmonids whereby the fish are adapted to life in fresh water during the autumn and winter and to life in the sea during spring and summer. Other physiological-behavioral parameters showing seasonal cycles in salmonids are: resistance to sea water (Conte and Wagner, 1965; Conte et al., 1966), salinity preference (Baggerman, 1960b; McInerney, 1964), thyroid activity (Baggerman, 1960a; Eales, 1963, 1965), resistance to a toxic substance (Alderdice, MS, 1963), and regulation of buoyancy (Pinder and Eales, 1969).

In the present study there were small differences in efficiencies of food conversion between groups of smolting salmon subjected to natural and reciprocal photoperiod. However, after entry into sea water there were clear reductions in efficiencies of food conversion in the natural and constant photoperiod groups, but a much greater reduction in the reciprocal photoperiod group. Later, after the fish were returned to fresh water, there was a sharp rise in efficiency in all groups to nearly identical levels. The lower efficiency of food conversion in the reciprocal photoperiod fish was clearly related to salinity. However, it was obvious that the poor growth of the reciprocal photoperiod fish was not due to low conversion efficiency alone since their feeding appetite was sharply reduced after they entered sea water. This shows that, in spite of there being no differences in osmotic and chloride levels among groups, salinity affected feeding appetite and growth patterns differently among groups.

Hoar (1965) points out the importance of photoperiod in the timing of the smolt transformation in salmonids and the associated cyclical changes in salinity tolerance and preference. The present study suggests that growth patterns during and after the smolting process are closely linked with, if not part of, the manifold smolting process and that both smoltification and the associated feeding and growth patterns can be modified by photoperiod.

Hormonal Control of Smoltification and Growth

A possible explanation for the observed effects of photoperiod is that the endocrine system, responding to stimuli via light-sensitive cells, produces hormones that act directly or through other glands to initiate and control the smolting process and associated osmoregulatory and growth patterns. The following observations on effects of growth hormone in various teleosts are offered as a possible explanation for differences in feeding appetite and growth of our salmon in respect to different photoperiod regimes. Swift and Pickford (1965) found cyclical changes in the growth hormone content of the pituitary gland of perch, *Perca fluviatilis*, with peaks in spring and early summer as determined from bioassay with hypophysectomized *Fundulus*. Swift (1954) found that injection of purified mammalian growth hormone stimulated growth of brown trout, *Salmo trutta*. Pickford (1957) says that hypophysectomized *Fundulus* respond to treatment with growth hormone with an obvious increase in appetite.

Some insight into the metabolic effects of growth hormone in fishes may be gained from studies with mammals. Russell (1955) suggests that growth hormone promotes synthesis of protein from amino acids and that fats supply the energy for this synthesis. Pickford (1957) says that growth hormone in higher vertebrates is generally considered to promote the mobilization of fats from storage depots to the liver and to accelerate fatty acid catabolism. Greenbaum (1955) stresses that formation of new protein in tissues is accompanied by disappearance of fat. It is highly speculative, but nevertheless attractive, to suggest that such processes are going on during smoltification and that a low level of growth hormone in our reciprocal photoperiod fish resulted in the opposite process from that described above, i.e., accumulation of fat rather than the reverse, which is the usual process during smoltification (Lovern, 1934; Malikova, 1957).

Although we did not demonstrate differences in osmoregulatory ability of salmon in respect to photoperiod, there was clearly a depressive effect of salinity on feeding and growth of fish subjected to a reciprocal photoperiod. Our food conversion efficiency data could be interpreted as suggesting that there was a greater metabolic cost of osmoregulation in sea water in the reciprocal than in the natural and constant photoperiod groups. It may be significant that Smith (1956) found that growth hormone increased salinity tolerance of brown trout.

There is evidence that the pituitary hormone prolactin has an osmoregulatory role in teleosts (Bern, 1967). Administration of prolactin prolongs the life of hypophysectomized *Fundulus* in fresh water (Pickford and Phillips, 1959) and is involved in the ability of sticklebacks (*Gasterosteus*) to adjust to fresh water (Lam and Hoar, 1967). Although much of the recent literature indicates that prolactin is involved in osmoregulation of fishes in fresh water and is of little importance in sea water (Ball, 1969), Dharmamba and Nishioka (1968) suggest that prolactin is not solely a "freshwater" hormone since the eta cells believed to produce prolactin appear to be present even in stenohaline marine teleosts. Moreover, Ball (1969) says that the prolactin cells in the pituitary gland of *Poecilia latipinna* are not totally regressed in dilute sea water as they are in full-strength sea water and he suggests that the action of prolactin is to reduce passive permeability of the integument, especially the gills, to sodium. Olivereau (1969) found that mitotic activity of prolactin cells continues in Atlantic and Pacific salmons and steelhead trout in sea water. Finally, prolactin may have among its many varied functions in teleosts (Bern, 1967) that of controlling fat deposition (Meier et al., 1966).

In their review of the literature on adrenocortical steroids, Chester Jones et al. (1969) show that these hormones are involved in regulating osmotic and metabolic levels in teleosts. The neurohypophysial peptides also seem to have a function in osmoregulation and seawater balance in teleosts (Perks, 1969).

These suggestions, that variations in production of growth hormone, prolactin, adrenocortical steroids, and neurohypophysial peptides are related to the observed differences in growth rate in response to photoperiod and to

salinity, are highly speculative. The role of the endocrine system in its control of the smolting process and of growth and osmoregulatory patterns during and after smoltification is little known. In view of the known and suspected functions of the above-named hormones, it should be profitable to follow changes in their production during the smolting process in salmon.

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NOTES

Marine Trematode *Tubulovesicula lindbergi* (Digenea: Hemiuridae) from Resident White Sturgeon in the Columbia River[1]

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BECKER, C. D. 1970. Marine trematode *Tubulovesicula lindbergi* (Digenea: Hemiuridae) from resident white sturgeon in the Columbia River. J. Fish. Res. Bd. Canada 27: 1313–1316.

An infection with a marine trematode, *Tubulovesicula lindbergi* (Layman, 1930) (Digenea: Hemiuridae), was discovered in a resident white sturgeon, *Acipenser transmontanus* Richardson, taken in the central Columbia River, Washington, USA. *Tubulovesicula lindbergi* is normally restricted to marine environs by virtue of essential (but still unknown) invertebrate intermediate hosts, but may occur in migratory fish that enter fresh water. Since hydroelectric dams on the Columbia are now effective barriers to the passage of sturgeon, it is postulated that *T. lindbergi* was transported into the river system by a maturing, anadromous teleost. After the postspawning death of the "transport" teleost host, the sturgeon presumably acquired the infection when scavenging. Consideration of basic synecological relationships supports the hypothesis. The white sturgeon is a new host record for *T. lindbergi*.

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SEVENTEEN SPECIMENS of *Tubulovesicula lindbergi* (Layman, 1930) Yamaguti, 1934, were recovered from a large (211-cm total length) white sturgeon, *Acipenser transmontanus*, taken in the central Columbia River above Richland, Washington, in early May 1969. This trematode (Digenea: Hemiuridae) is normally a stomach inhabitant of marine teleosts and is notably lacking in host specificity, a trait characteristic of many hemiurids. *Tubulovesicula lindbergi* has been reported from several diverse species of marine fishes taken along the coasts of Oregon, Washington, and British Columbia (Ching, 1960; McCauley, 1960; Pratt and McCauley, 1961; Margolis, 1963; Arai, 1969). Although *T. lindbergi* occurs along both the eastern and the western rims of the North Pacific Ocean, its geographic distribution is apparently not continuous for it does not appear in fishes from far northern waters (Skrjabin and Gushanskaya, 1954; Mamaev et al., 1959; Zhukov, 1960; Mamaev and Oshmarin, 1963).

Correct identification in cases of unusual parasitological records is prerequisite to further consideration. The morphology of *T. lindbergi* tends to

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be highly variable, resulting in considerable synonymity (McCauley, 1960), and thus requires close scrutiny. My presumptive identification, based on study and measurements of preserved and mounted specimens, was confirmed by Dr J. E. McCauley, Oregon State University.

The presence of a "marine" trematode in a "freshwater" teleost is not, in itself, a rare phenomenon. In known records the definitive hosts are usually anadromous, catadromous, or diadromous fishes that migrate between freshwater and marine habitats, or euryhaline fishes that habitually dwell in estuaries of varying degrees of salinity. The internal microenvironment of the teleost host provides protection from changes in the external macroenvironment, which would otherwise be lethal to the parasite. Features of the aquatic macroenvironment, such as salinity, however, rigidly limit the geographic range of invertebrate intermediate hosts, which are essential for completion of a trematode's life cycle. Similar features are usually pernicious to free-swimming larval stages, the miracidia and cercaria. For these reasons, the occurrence of *T. lindbergi* in a sockeye salmon (*Oncorhynchus nerka*) taken from Lake Washington (near Seattle) was considered to be ipso facto evidence that the fish had been to sea (Margolis, 1965).

English translations of two Russian texts (Bykhovskaya-Pavlovskaya et al., 1962; Dogiel et al., 1958) state that *T. lindbergi* has been *introduced into* Soviet fresh waters with euryhaline fish ("plaice" and sturgeon). Dr L. Margolis (personal communication) pointed out that an accurate translation would read *carried into* rather than *introduced into* (my italics). The former merely indicates that the parasite was carried with a teleost host moving from salt to fresh water, whereas the latter implies that the parasite had become established in a freshwater habitat. Precise references to the statements were not given in either text, and therefore the original sources remain unknown to me. One later paper (Akhmerov, 1961) reports *T. lindbergi* in anadromous chum salmon (*Oncorhynchus keta*) and sturgeon (*Huso dauricus*) from the Amur River, USSR.

Formulation of a hypothesis to explain the occurrence of a marine trematode in a fish restricted to freshwater environs requires consideration of the basic synecological relationships existing between the parasite, its invertebrate intermediate hosts, its teleost definitive host, and the aquatic habitat of these hosts. The following facts seem to be relevant to the question.

Tubulovesicula lindbergi has low phylogenetic specificity for teleost definitive hosts, as indicated by numerous infection records, but the first and second intermediate hosts — presumably a marine mollusc and an arthropod — have not been identified. Few data are available on the parasite's life cycle. However, it has been demonstrated that individuals infecting chum salmon mature in 2–4 months, and that they can live for at least 31 months while growing from about 0.6 to more than 4.5 mm in length (Margolis and Boyce, 1969).

White sturgeon in the Columbia River, although inherently capable of surviving in both fresh and salt water, formerly displayed restricted mi-

gratory habits and did not ordinarily enter the sea (Bajkov, 1951). Hydroelectric dams constructed on the lower Columbia (i.e., Bonneville in 1938, McNary in 1953, The Dallas in 1957, and John Day in 1968) now serve as effective barriers that prevent the upstream and downstream movement of sturgeon (U.S. Corps of Engineers, 1968). Moreover, white sturgeon are omnivorous grazers and are known to scavenge on the remains of dead fish (Bajkov, 1951; Semakula and Larkin, 1968). Presumably the larger the scavenger, the larger the carcass it can ingest.

Anadromous fish that surmount dams via fishways and penetrate some 300 river miles into the central Columbia include chinook (*Oncorhynchus tshawytscha*), coho (*Oncorhynchus kisutch*), and sockeye (*O. nerka*) salmon, steelhead trout (*Salmo gairdneri*), shad (*Alosa sapidissima*), and Pacific lamprey (*Lampetra tridentata*) (U.S. Corps of Engineers, 1968). Except for steelhead, these species inevitably die after spawning and become available to scavenging fish such as sturgeon. Among these potential transport hosts, salmonids are frequently recorded as hosts of *T. lindbergi* when dwelling in the sea (Zhukov, 1960; Ching, 1960; McCauley, 1960; Akhmerov, 1961; Mamaev and Oshmarin, 1963; Margolis, 1963; Margolis and Boyce, 1969).

According to Dr L. Margolis (personal communication), *T. lindbergi* has been found in prespawning anadromous salmon collected in British Columbia several hundred miles from salt water. The parasite can, therefore, survive for days (possibly even weeks) in fasting salmon after they enter fresh water. Although dams have now inundated many pristine spawning areas for salmonids in the Columbia system, a 93-km section of the central Columbia still supports sizable spawning populations of anadromous fish. The parasitized sturgeon reported herein was taken in this area.

Recapitulation of these data suggests the feasibility of the following events. Normally restricted to marine environs by its essential (but still unknown) intermediate hosts, *T. lindbergi* was carried into the central Columbia River by an anadromous fish, likely a Pacific salmon (genus *Oncorhynchus*). After spawning in the fall, the moribund transport host or its carcass was ingested by the resident sturgeon, which thus acquired the infection. The known life span of this hemiurid (at least 31 months) covers the duration of a lengthy upstream migration of an anadromous fish, its postspawning death, and subsequent survival of the parasite in sturgeon until captured in early May. (Of the recovered specimens, one was still immature.) Dr J. E. McCauley (personal communication) noted that the hardy stomach parasite has only a short migration from the stomach of a prey fish to the stomach of a predator or scavenger fish; unlike an intestinal parasite, it does not have to pass through a hostile environment but finds itself in an environment essentially similar to the one just left.

Support of the hypothesis is provided by records of *T. lindbergi* in returning adult sockeye collected at Cellilo Falls (now inundated) on the lower Columbia River (Margolis, MS, 1956). Anadromous fish undoubtedly carry this marine trematode into the central Columbia and into its major tributary, the Snake

River. The hypothesis, however, precludes *T. lindbergi* from completing its entire life cycle in a freshwater habitat because of the absence of essential intermediate hosts and survival of temporary free-living stages.

The white sturgeon, *A. transmontanus*, is a new host record for *T. lindbergi*.

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Metazoan Parasites, Excluding Monogenea, from Longnose and White Suckers[1]

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THRELFALL, W., AND G. HANEK. 1970. Metazoan parasites, excluding Monogenea, from longnose and white suckers. J. Fish. Res. Bd. Canada 27: 1317–1319.

Seven genera of helminths (two of Digenea, one of Cestoda, and two each of Nematoda and Acanthocephala) and one species of parasitic copepod were recovered from 40 longnose suckers (*Catostomus catostomus*) and five white suckers (*Catostomus commersoni*), caught at two localities in Labrador, during August and September 1969. The helminths reported include five new host records.

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FEW OF THE MANY PAPERS written about the parasites of freshwater fishes in North America (Hoffman, 1967) are concerned with fishes from the more northerly regions of the continent. Little research has been done on the metazoan parasites of animals living in the Labrador area. Munroe (1949) recovered cestodes, nematodes, and acanthocephalans from specimens of *Salvelinus fontinalis* taken in the interior of Labrador, and Andrews and Lear (1956) found cestodes and nematodes in *Salvelinus alpinus* from the coastal regions of northern Labrador.

Longnose suckers (*Castostomus catostomus*) and white suckers (*Catostomus commersoni*) are found in freshwater lakes, rivers, and streams in North America (Eddy, 1957), both species being hosts for a wide range of parasites (Hoffman, 1967). Low (1896) stated that both species were common in Labrador, and Backus (1957) collected specimens, from the eastern part of the region, during the *Blue Dolphin* Labrador Expeditions of 1949, 1950, and 1951. A survey was, therefore, initiated in the summer of 1969 to determine the parasite burden, if any, of suckers from eastern Labrador.

Materials and methods — Fifteen longnose suckers caught in Eagle River, Labrador (53°-34′N, 57°27′W), and a further 25 longnose and five white suckers from Grand Lake, Labrador (53°35′N, 60°15′W), were examined for metazoan parasites during August and September 1969. The fish were caught in either a 30-ft hand seine or gillnets, and were examined immediately upon capture.

Digenetic trematodes, cestodes, and acanthocephalans were fixed and stored in 5% formalin and stained afterwards with Semichon's acetic carmine and mounted in Canada balsam. Nematodes were treated in the manner described by Hanek and Threlfall (1970); parasitic copepods were fixed and stored in either 5% formalin or 70% alcohol and examined as wet mounts in one of these media.

Monogenetic trematodes, dealt with elsewhere, were also recovered from the fish.

Specimens of all the parasites found have been deposited in the collection of the Department of Biology, Memorial University of Newfoundland, St. John's, Nfld.

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Results and discussion — Twenty-nine longnose suckers (72%) and four white suckers (80%) were found to be infected with metazoan parasites. Seven genera of helminths and one species of parasitic copepod were recovered from the specimens (Table 1). Fewer species of parasites were present in the white

TABLE 1. Infection of *Catostomus catostomus* and *Catostomus commersoni* with metazoan parasites, excluding monogenetic trematodes. Range per infected fish in parentheses.

Species	Host[a]	Locality[b]	Location	No. examined	Infected (%)	Avg no. per infected fish
Lissorchis gullaris Self and Campbell, 1956	A	*	Intestine	15	20	11.5(3–19)
Diplostomulum spathaceum (Rudolphi, 1819)	A	**	Eye lens	25	32	13.8(7–25)
	B	**	"	5	20	10.0
Diplostomulum sp.	A	*	"	15	13	3.0(2–4)
Hunterella nodulosa Mackiewicz and McCrae, 1962	A	*	Intestine	15	20	2.3(1–4)
	A	**	"	25	32	3.6(1–9)
	B	**	"	5	60	3.0(1–7)
Proteocephalus sp.	A	**	"	25	4	4.0
Contracaecum sp.	B	**	"	5	20	5.0
Rhabdochona sp.	A	*	"	15	53	2.6(1–7)
	A	**	"	25	12	1.3(1–2)
	B	**	"	5	20	2.0
Neoechinorhynchus cristatum Lynch, 1936	A	**	"	25	20	3.6(2–9)
Neoechinorhynchus strigosum Van Cleave, 1949	A	**	"	25	16	4.2(2–7)
	B	**	"	5	20	3.0
Ergasilus caeruleus Wilson, 1911	A	**	Gills	25	24	1.5(1–3)
	B	**	Gills	5	20	1.0
Total	A	*		15	87	16.8(1–30)
	A	**		25	64	6.0(1–29)
	B	**		5	80	7.0(2–10)

[a]A = *Catostomus catostomus*; B = *Catostomus commersoni*.
[b]* = Eagle River; ** = Grand Lake.

than the longnose suckers (6:9, respectively), this difference perhaps being attributable to the difference in sample size of the two species (5:40).

Lissorchis gullaris was described from buffalo fish caught in Lake Texoma, Oklahoma (Self and Campbell, 1956). The present specimens, from *C. catostomus* (new host record), fitted the original description in all respects except for the shape of the testes, which were lobed (six to eight lobes/testis), those of the holotype being ovoid. Ulmer (1950) showed that the total size of specimens of *Postharmostomum helicis* from *Peromyscus maniculatus* could be changed by flattening the organisms and that in nonmuscular organs, such as the testes,

flattening of specimens could radically alter the shape of the organs. Flattening the trematodes often resulted in distinct lobation of the testes, which may be the situation obtaining in the present work. Ulmer (1951) also showed that an increase of almost 100% in the size of the testes might result from extreme flattening.

Diplostomulum spathaceum has been recovered from eye lenses of a wide variety of fishes in North America and Europe (Hoffman, 1967), but has never previously been recovered from *C. catostomus* and *C. commersoni*; Bangham and Adams (1954) noted the presence of a *Diplostomulum* sp. in these hosts in British Columbia.

Mackiewicz and McCrae (1962) described *Hunterella nodulosa* from *C. commersoni* taken in 14 states of the USA. The presence of this helminth in *C. catostomus* is a new host record, as is the presence of *Proteocephalus* sp.

All the nematodes found were immature; consequently, no specific name was assigned to them. Both species of Acanthocephala, and *Ergasilus caeruleus*, have been recovered from one or both of these hosts (Hoffman, 1967).

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Sporulation of a Haplosporidan in a Pacific Oyster (*Crassostrea gigas*) in Humboldt Bay, California[1]

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KATKANSKY, S. C., AND R. W. WARNER. 1970. Sporulation of a haplosporidan in a Pacific oyster (*Crassostrea gigas*) in Humboldt Bay, California. J. Fish. Res. Bd. Canada 27: 1320–1321.

A Pacific oyster (*Crassostrea gigas*) with large numbers of acid-fast spores was collected from Humboldt Bay, California, in August 1969. The spores were tentatively identified as belonging to a haplosporidan of the genus *Minchinia*. This is the first report of sporulation of a haplosporidan in a Pacific oyster from the United States.

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THE PRESENCE of haplosporidans in oysters and their effects are well known. *Minchinia nelsoni* has been identified as the etiological agent of oyster epizootics on the middle Atlantic coast of the United States (Haskin et al., 1966). A similar sporozoan, *Minchinia costalis*, has been associated with other oyster epizootics on the Atlantic coast (Wood and Andrews, 1962).

Routine examination of a gaping Pacific oyster collected from Humboldt Bay, California, in August 1969 revealed it to be heavily infected by operculated spores (Fig. 1), which were tentatively identified as belonging to a haplosporidan of the genus *Minchinia*. Subsequent staining of fixed material by a technique described by Farley (1965) showed these spores to be acid fast. Dr V. Sprague of the University of Maryland confirmed our diagnosis, and indicated this haplosporidan was probably an undescribed species. Sporulation resembled that of *M. costalis* in that it occurred in the leydig tissue; however, the spores appear intermediate in size between *M. nelsoni* and *M. costalis*.

The oyster in which the spores were observed was transplanted to Humboldt Bay from Pendrell Sound via Denman Island, B.C., Canada, in March 1966 when less than 1 year old. Plasmodial stages (but no spores) of a haplosporidan were noted a few years ago in a Pacific oyster from Denman Island in Canada (V. Sprague, personal communication). It is not known at this time if this parasite was transferred to Humboldt Bay from Pendrell Sound or Denman Island or if low levels of infection exist naturally in these areas.

Mass mortalities of Pacific oysters have occurred in recent years in Humboldt Bay. Histological examination of live and dead oysters during the period of mortality did not implicate haplosporidans as the cause.

[1]This study was made in conjunction with investigations by research contract, No. 14-17-0001-1382, with the Bureau of Commercial Fisheries, U.S. Fish and Wildlife Service.

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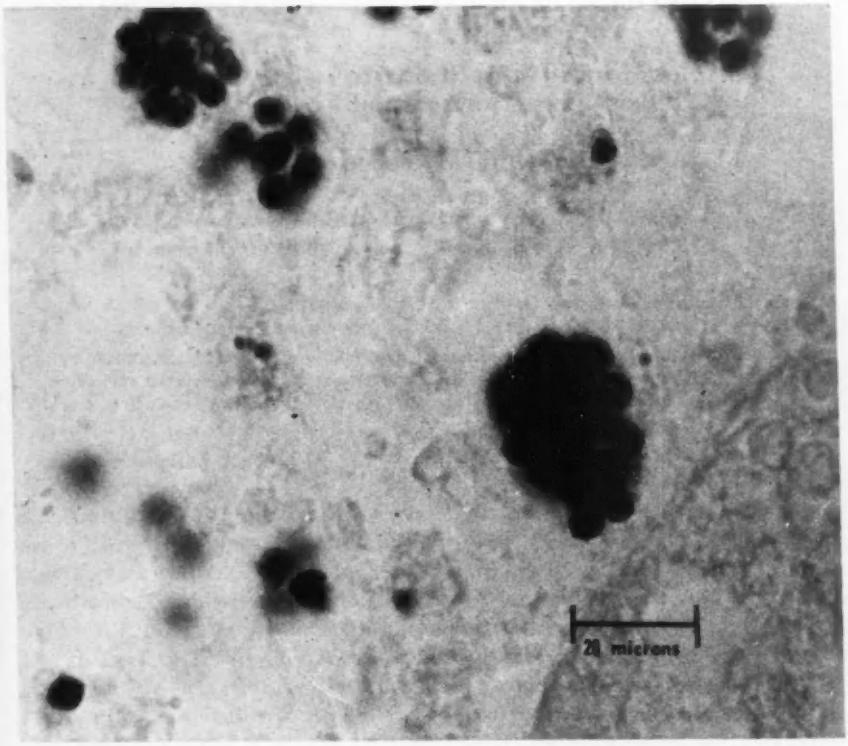

FIG. 1. Haplosporidan spores from a Pacific oyster (*Crassostrea gigas*).

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A Modified Version of the Blazka Respirometer and Exercise Chamber for Large Fish[1]

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SMITH, L. S., AND T. W. NEWCOMB. 1970. A modified version of the Blazka respirometer and exercise chamber for large fish. J. Fish. Res. Bd. Canada 27: 1321–1324.

A tube-within-a-tube respirometer has been built in which a jet outboard impeller propels the water. A towline attached to the fish through its dorsal musculature to keep it out of the pump avoids the electrolysis problems of an electric grid when using sea water.

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RESPIROMETERS OR EXERCISE CHAMBERS FOR FISH in which water is pumped are of three basic types: small volume with low horsepower, small volume with high horsepower, and large volume with low horsepower. Each differs from the other in the combination of requirements for horsepower, frictional loss (pumping head), and water volume. A respirometer with small water volume provides the greatest sensitivity for measurement of changes in oxygen consumption, but one with large volume gives the least turbulent flow at lowest head. Diffusion grids can enable control of the flow pattern in a small-volume respirometer, but the screens require increased horsepower to overcome the added friction. The use of low horsepower may minimize both construction and operating costs as well as the heat input into the system but usually requires the sacrifice of uniform cross-sectional velocity and results in a very bulky apparatus. Thus the three combinations of features are mutually exclusive, engineering compromises in which each desired feature is obtained only at the expense of another.

Designs of the three types of respirometers are shown in Fig. 1. Brett's respirometer (Mar, 1959; Brett, 1964) can be characterized by high horsepower, high head, and low volume. Burrow's stamina tunnel (Thomas et al., 1964;

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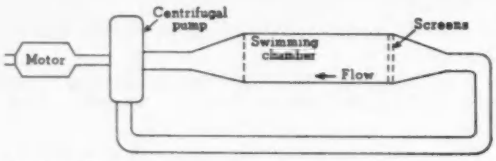
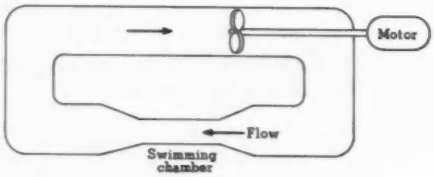
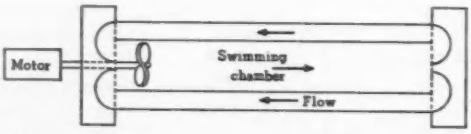


A. Brett's respirometer



B. Burrow's stamina tunnel as modified by Parker



C. Blazka's respirometer

FIG. 1. Types of closed respirometers for fish.

R. Parker, personal communication) has such a large volume that it is used mostly as an exercise chamber but has relatively uniform velocity across the tunnel diameter without the use of screens. Therefore it has a low head and requires minimal power. Blazka's respirometer (Blazka et al., 1960) has minimal volume, minimal head, and minimal power, but probably has the poorest flow profile and is the most difficult of the three types to get fish into and out of. Other respirometers (Ivlev, 1962; Katz et al., 1959) are hybrids of these three types and are not widely used. Reported in this paper is a respirometer and exercise chamber that is a modification of the Blazka-type respirometer. It has proved to be particularly advantageous for physiological studies of large salmon aboard the Fisheries Research Institute's new floating laboratory, the R.V. *Kumtuks*.

Design and construction — Our version (Fig. 2) was made with two plexiglass (acrylic) tubes 12 inches (30.5 cm) and 8 inches (20.3 cm) in diameter with walls ¼ inch (6.6 mm) thick. The smaller tube is loosely centered inside the larger tube by four vanes at each end. A removable set of vanes inside the smaller tube at the upstream end controls direction of flow. Flanges were machined from flat plexiglass plate and glued to the outer tube for attachment of the end blocks and for mounting of the tubes on a plywood base. The end blocks were laminated from lumberyard hardwood, turned on a wood lathe, and fiberglassed for waterproofing. The shape shown for the end blocks has proved sufficiently satisfactory so that we plan to have them duplicated in cast aluminum. The plywood base is supported on an axle and a framework that allow the whole respirometer to be tilted for insertion of fish into the respirometer while it is partly filled with water.

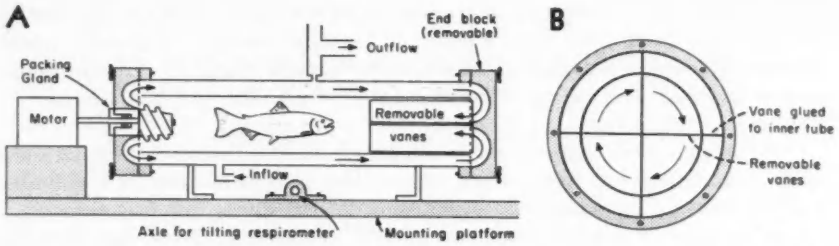


FIG. 2. Design of respirometer and exercise chamber, showing (A) side and (B) end views.

Partly filling the respirometer in the tilted position minimizes the amount of time that the fish is out of water. A ¾-hp motor with a variable-speed magnetic clutch drives a jet outboard impeller mounted on a stainless steel driveshaft. The driveshaft passes through the end block in a standard, graphited, packing gland. The jet outboard impeller causes less spiral movement of the water than a propeller or centrifugal impeller would, and produces a flow having very low turbulence and velocities up to 131 cm/sec. When the experimental fish fills a significant portion of the cross-sectional area of the chamber, the effective velocity is considerably greater, depending on the size of the fish. Water enters the respirometer at the bottom and leaves from a large-diameter, low-turbulence standpipe at the top. Tubes, wires, and other attachments to the fish also exit there.

Operation — An anesthetized fish is put into the partly filled respirometer through the end opposite the impeller. Any tubes, wires, or other attachments are pulled through the standpipe and a hole in the inner tube with a hooked wire. The inner vanes are inserted, the end block is secured with wing nuts, and the respirometer leveled. The impeller is rotated while the respirometer completes filling so that the water movement provides a respiratory current for the fish and induces recovery from the plane III (respiratory arrest) level of anesthesia used during surgery. Between 2 and 24 hr of recovery in the respirometer are allowed before an experiment is begun, the length of time depending on the experimental requirements.

The reverse procedure is followed for removal of the fish from the respirometer. The respirometer is tilted, the water level lowered, and the end block removed. Either the fish is pulled out by its tubes (after being disconnected from the recorder, etc.) or the respirometer is tilted down again and the remaining water with the fish poured into a large container.

Controlling the position of the fish in the respirometer without interfering with free swimming is a continuing problem. Several respirometers use screens or grids downstream from the fish to keep the fish out of the pump and provide electrification of a grid to stimulate swimming. However, we wished to minimize frictional losses and electrolytic products in sea water are toxic; therefore screens were not included in the design. We had already found that small adult salmon preferred being towed by a cannula anchored in their nasal cartilage rather than swimming against the pumped stream of water. Preliminary trials in this respirometer showed that most fish will swim either when the water velocity increases or when their tail touches the rotating impeller. However,

larger fish break, pull out, or damage most of the tubing and routine attachments. Thus we have inserted a heavy nylon cord through the muscles under the anterior edge of the fish's dorsal fin — the approximate node of its swimming oscillations and also its approximate center of gravity. We call this cord a "tether" or "towline."

In use, the towline is led out of the standpipe and tied with minimal slack while the fish recovers from surgery or previous periods of exercise. Swimming is stimulated by increasing the water velocity and giving the fish more slack in the towline. If needed, sufficient slack is provided to allow the fish's tail to touch the impeller. Brightly illuminating the downstream half of the respirometer and darkening the upstream half help the fish to learn to hold position in the swimming chamber. The towline system has been used successfully on salmonids ranging in weight from 0.3 to 10 kg for up to 2 weeks. The towline does not seem to cause necrosis or promote infection at the puncture site, even in the presence of the fungus *Saprolegna* elsewhere on the fish. Fish become accustomed to the towline usually within minutes. Because water flow produced by the impeller is sufficient only to meet the fish's respiratory needs, fish have an extremely low level of ventilatory activity while they are being towed.

Acknowledgments — We acknowledge the contribution of Dr F. E. J. Fry, who stimulated our thinking about a Blazka respirometer with an adaptation of his own. Also, we acknowledge the technical assistance of machinist F. Rolands of the University of Washington, College of Fisheries, and P. Swierkowski, Chief Engineer of the *Kumtuks*.

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Existence in Salmonid Hemoglobins of Molecular Species with Three and Four Different Polypeptides

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TSUYUKI, H., AND A. P. RONALD. 1970. Existence in salmonid hemoglobins of molecular species with three and four different polypeptides. J. Fish. Res. Bd. Canada 27: 1325–1328.

The approximate equivalence of tryptic fragments and total basic amino acids per molecule of hemoglobin in five species of Pacific salmon, and the demonstration in sockeye salmon (*Oncorhynchus nerka*) of three or four electrophoretically distinct polypeptides in each of six major hemoglobin fractions separated by starch-gel electrophoresis, provided strong evidence for the in vivo existence in salmonids of molecular species of hemoglobins consisting of three and four different polypeptides. At least eight electrophoretically distinct polypeptides, including forms allelic to both the α- and β-type proteins, were found, accounting for the presence of large numbers of molecular species of hemoglobins and providing further evidence for the tetraploid origin of most salmonids.

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IN HIGHER VERTEBRATES, hemoglobins (Hb) typically possess a tetrameric structure consisting of two α and two β or β-like chains, each with a heme group attached (Braunitzer et al., 1964). Deviations from this general structure are found in certain abnormal human hemoglobins. For example, the globin in HbH consists only of four β chains (Jones et al., 1959) and Hb "Barts," the fetal counterpart of HbH, consists of four chains (Hunt and Lehmann, 1959; Kekwick and Lehmann, 1960). Huehns et al. (1961) described a hemoglobin with only one α polypeptide capable of existing as a molecular entity.

Recently, a hybrid hemoglobin molecule with three kinds of polypeptide chains was reported (Manwell and Baker, 1967) from a study of turbot (*Scophthalmus maximus*) hemoglobin that existed in polymorphic forms consisting of a single slow zone, a single fast zone, or three zones. The hybrid hemoglobin, which also formed spontaneously from a mixture of slow and fast components, was shown to consist of two α and one each of β^F and β^S chains. A two-dimensional chromatography of the tryptic digest of the fetal and adult hemoglobins of the spiny dogfish, *Squalus suckleyi*, showed the presence of 43 and 41 ninhydrin positive spots respectively, and suggested the existence of at least two different kinds of polypeptide chains (Manwell, 1963).

A total of 58 basic (lysine and arginine) amino acid residues per mole has been reported for the fast hemoglobin component of rainbow trout, *Salmo gairdneri* (Buhler, 1963). Corresponding values of 59 and 55 were reported for the fast hemoglobins of chinook salmon, *Oncorhynchus tshawytscha* (Buhler, 1963) and for chum salmon, *O. keta* (Eguchi et al., 1960). The fast hemoglobins

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of each of the five species of *Oncorhynchus* from the northeastern Pacific Ocean has subsequently been separated by starch-gel electrophoresis into several components migrating anodally at *p*H 8.5 (Tsuyuki and Gadd, 1963; Tsuyuki et al., 1965; Yamanaka et al., 1967). In our study, the number of basic residues of the several anodic components of each species ranged from 54 to 58 for sockeye salmon (*O. nerka*), 51 to 54 for coho salmon (*O. kisutch*), 57 for chum salmon, 58 to 61 for pink salmon (*O. gorbuscha*), and 55 to 58 for chinook salmon. These values compare with 56 found in human HbA and with other mammals that show analyses in the same range.

When the peptide maps of tryptic digests of anodic components were examined, the five species of Pacific salmon showed a total of 66 clearly defined ninhydrin positive areas (Fig. 1) ranging from 56 to 58 in sockeye salmon,

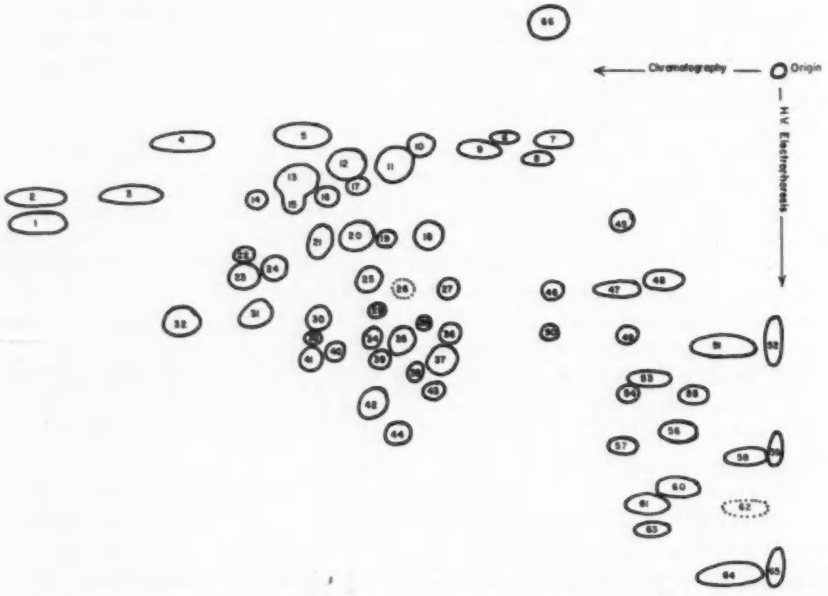

FIG. 1. A combined peptide map of the tryptic digests of the hemoblobins from five species of Pacific salmon.

47 to 48 in coho salmon, 44 to 52 in chum salmon, 48 to 55 in pink salmon, and from 47 to 50 in chinook salmon. The number of ninhydrin positive areas in each anodic fraction of each species, as well as the intra- and the inter-specific differences, were repeatedly confirmed. Furthermore, the number of ninhydrin positive areas coincide approximately with the total number of basic amino acid residues calculated, assuming a molecular weight of 68,000, based on iron analysis. This represents approximately double the theoretically possible 28 spots (Ingram, 1958) from the tryptic digests of human HbA,

consisting of 14 from the α chains and 14 from the β chains, and four times the number of spots observed from the digests of the abnormal human HbH (Jones et al., 1959), which consists of 4β chains only.

Further studies on sockeye salmon revealed the presence of three and four constituent polypeptides from each of the three major anodal and three major cathodal zones obtained by starch-gel electrophoresis of hemoglobins at *p*H 8.5. All three cathodal molecular species yielded the same two different α-type polypeptides and either one or both of the two different β-type polypeptides, all as major components. All three anodic fractions of sockeye salmon hemoglobin possessed one electrophoretically identical α-type polypeptide and either one or two of what appear(s) to be three electrophoretically distinct β-type polypeptides appearing as major components. These observations are in contrast to the two major polypeptides reported in *Salmo salar* (Wilkins, 1968). None of the polypeptides from the cathodal fractions possessed the same mobility as those from the anodal fractions. At least eight different structural genes are therefore coding for the polypeptides of the major hemoglobins of sockeye salmon. The number of tryptic fragments observed in the anodal hemoglobins indicate that the single α-type polypeptide may consist of two difficultly separable polypeptides.

On the basis of approximate equivalence of total basic amino acids and tryptic fragments in some of the molecules, the actual demonstration of three and four different polypeptides per molecule, absorption spectra, and iron analyses, we propose that in the Pacific salmon and other salmonid species the hemoglobins exist, in the native state, as tetrameric structures comprised of four and, in some instances, three different polypeptides in addition to the usual complement of four heme groups. This is in marked contrast to the generally accepted view of mammalian hemoglobins that typically possess a tetrameric structure consisting of 2α and 2β chains.

Further support for such a structure could be found in the lactate dehydrogenase (Klose et al., 1967; Ohno et al., 1968; Massaro and Markert, 1968) and malate dehydrogenase (Bailey et al., 1969) systems of salmonids, which provide evidence that the whole genome has indeed duplicated and that these species are, therefore, tetraploid — a hypothesis first advanced by Ohno and Atkin (1966) and Ohno et al. (1968) on the bases that these fishes have approximately twice the DNA content per nucleus and twice as many chromosomes as the *Clupeiformes*. The presence in both the cathodic and anodic hemoglobins of what appear to be proteins allelic to the α and β polypeptides and, therefore, capable of replacing, in the natural state, the respective functions of the two types of globins, constitutes further evidence for tetraploidy of salmonids. The first report (Efremov et al., 1969) of successful in vitro hybridization of hemoglobin Richmond with other human hemoglobin variants to produce an asymmetric hybrid consisting of four different polypeptides also support our proposals for the structure of salmonid hemoglobins. A more detailed study will be reported later.

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Further Studies on Scallop Sterols in Relation to Cholesterol in the Chick

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The blood cholesterol concentration of chicks fed a cholesterol-supplemented diet decreased significantly ($P < 0.05$) when scallop sterols were added to the diet. There was no significant change in plasma Δ^5-sterol when scallop sterols were incorporated into the basal diet.

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THE ELEVATION of blood cholesterol induced in the chick by a diet containing 0.5% added cholesterol was insufficient to establish the significance of a depression that resulted from the addition of 0.5% scallop sterols (Idler et al., 1970). The experiment was repeated with 1% added sterols.

Methods — Day-old leghorn cockerels were fed the basal diet ad libitum for 1 week and weighed. Then chicks were assigned to 12 experimental units of four chicks each so that average body weight for each set of four chicks was approximately equal. The diets were assigned at random to the experimental units. Dietary treatments were basal, and basal supplemented with 1% of cholesterol, or scallop sterols, or both. On the 14th day of test-feeding, jugular blood samples were taken from each chick.

Plasma cholesterol concentration was measured by the method of Sperry and Webb (1950). Gas–liquid chromatography (GLC) was used to check the cholesterol content of the scallops and to analyse the chick plasma for sterols other than cholesterol (Idler and Wiseman, 1968).

Results — Plasma cholesterol levels resulting from feeding the sterols are shown in Table 1. The addition of 1% cholesterol to the basal diet produced a highly significant increase in the plasma cholesterol level (Table 2). The treatment interaction was also highly significant. There was no significant increase in plasma cholesterol level when scallop sterols were added to the basal diet (confidence interval 8 ± 15 at the 95% level of significance). The chicks fed cholesterol plus scallop sterols had plasma cholesterol levels significantly lower than those fed cholesterol (confidence interval 28 ±15 at the 95%

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TABLE 1. Plasma cholesterol levels of chicks fed sterol-supplemented diets.

	Basal[a] diet	Basal + 1% cholesterol	Basal + 1% scallop sterols	Basal + 1% scallop sterols + 1% cholesterol
No. chicks	12	12	12	12
Plasma cholesterol (*mg/100 ml*)[b]	127±24	162±41	135±31	134±16
Mean food consumption (*g/chick per day*) days 8–21	22.9	22.9	21.9	22.7
Mean wt gain (*g/chick*) days 8–21	138	138	132	136

[a]Cornmeal, 45%; ground wheat, 14%; stabilized tallow, 2%; soybean meal, 35%; ground limestone, 1.5%; dicalcium phosphate, 1.5%; iodized salt, 0.5% and micronutrients, 0.5%.

[b]Mean ± 1 SD. Standard deviation within treatments σ_i estimated as follows:

$$(\sigma_i^2 = \tfrac{1}{8} \sum_{i=1}^{12} (x_i - \bar{x}_u)^2$$

where x_i is the result for the ith individual and $\bar{x}_u$ is the mean of its experimental unit.

TABLE 2. Analysis of variance of the effects on plasma cholesterol of incorporating cholesterol and scallop sterol into a chick diet.

Source	df	Mean square	F	*P*
Cholesterol	1	867	12.7	< .005
Scallop sterol	1	299	4.3	.05< .10
Cholesterol X Scallop sterol	1	989	14.5	< .005
Error	8	68.2		

level of significance). The mean values of plasma cholesterol were 127 mg/100 ml for the chicks on the basal diet, 134 for the basal diet with both cholesterol and scallop sterols, 135 for tho basal diet with scallop sterols, and 162 for the basal diet with cholesterol.

The scallop sterols contained 25% cholesterol by GLC. There were no additional sterols found in the plasma of chicks fed with scallop sterols.

Discussion — When cholesterol plus scallop sterols or scallop sterols alone were fed, the plasma cholesterol level was significantly decreased compared to the level when cholesterol alone was fed, even though cholesterol was a significant component (25%) of scallop sterols.

When the sterols were added to the basal diet at the 0.5% level, the depression of serum cholesterol produced by feeding scallop sterols was not significant at the 95% level (Idler et al., 1970). The elevation in serum cholesterol

produced by feeding 0.5% cholesterol in the diet was 17%. In the present experiment, elevation of plasma cholesterol by exogenous cholesterol was 28%, much lower than that reported in previous experiments (Reiner et al., 1960, 1962). The breed of chick, or the diet, or both, will now be varied in order to establish conditions where cholesterol will produce a greater elevation of plasma cholesterol.

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